MEASUREMENT OF AIRWAY BLOOD FLOW BY LASER DOPPLER FLOWMETRY

by

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DECLARATION

The studies described in this thesis were performed in the University of British Columbia, Pulmonary Research Laboratory, St Pauls Hospital, Vancouver, B.C., Canada, and in the Department of Medicine, Francis Scott Key Medical Centre, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland, U.S.A. I was responsible for the design of the experiments, in conjunction with Professor P.D. Paré and Ms. E.M. Baile M.Sc. I took part in the surgical preparation of animals, in conjunction with Ms. Baile and Dr. E.M. Wagner. All laser Doppler flow measurements in animals and humans were made by myself.

I undertook all of the data analysis, and prepared all figures and tables. This thesis has been composed and typed by myself.

Signed

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ABSTRACT OF THESIS

Laser Doppler flowmetry (LDF) is a non-invasive method of measuring microcirculatory blood flow based on quantifying the Doppler frequency shift imparted to laser light by moving red blood cells. The object of this study was to examine whether a laser Doppler flow probe could be used to measure airway wall blood flow during bronchoscopy. Studies were performed in dogs, sheep and humans.

Artifactual LDF flow signals were identified due to inadequate contact between the probe head and the mucosa, ventilatory and cardiac movement, and ambient light interference. Measurement technique was modified to minimise these artifacts.

Site-to-site variation in LDF flow signals under baseline conditions was observed in all species (mean coefficient of variation = 36%), and, in humans, variation was similar in awake subjects during breath-holding, and in anaesthetised subjects during cardiopulmonary bypass, in whom ventilatory and cardiac artifacts are absent.

When the probe was held at a single site, linear flow-pressure relationships ($r = 0.63 - 0.9$, $p<0.001$) were observed in the trachea in 7 dogs during acute changes in mean systemic blood pressure and airway pressure. In 4 sheep, average LDF flow signals within regions of the bronchi varied in a linear fashion with changes in blood flow through a cannulated bronchial artery perfused by a roller pump. However, site-to-site variation in response occurred, and a substantial signal persisted when bronchial arterial flow was stopped, or when the artery was perfused by a cell-free solution of dextran which would, in theory, be expected to produce no LDF signal. These results may be explained in part by collateral blood flow, but also indicate detection of "noise".

In 5 dogs, blood flow was measured in 6 regions of the trachea by both LDF and by the radiolabelled microsphere reference flow technique during resting ventilation (baseline), application of 15 cm H_2O positive end-expiratory pressure (PEEP) and during hyperventilation of dry air (HV). When regional measurements were examined, weak, but significant correlations were observed between LDF and reference flow measurements during each condition. However, during PEEP, although both techniques indicated a similar mean reduction in blood flow (63%) from baseline, there was no correlation between the techniques in the magnitude of reduction measured in individual regions. During HV, LDF measurements showed variable responses between regions, and the mean change from baseline was not significantly different from zero. In contrast, reference flow values increased in most regions, and the mean increase was 87% from baseline. Sectioning of the tracheal wall indicated that this increase was localised to the mucosal layer.

The results indicate that acute changes in blood flow at single sites in the airway may be detected by LDF applied in the present fashion. However, detection of "noise" and site-to-site variation in LDF signals preclude quantitative measurements of airway wall blood flow using this probe design, particularly when the probe is moved between sites during an intervention.

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UNITS

Quantity	Units	Symbol
Length	millimetres	mm
	centimetres	cm
Mass	microgram	ug
	milligram	mg
	gram	g
	kilogram	kg
Temperature	Degrees Centigrade	°C
Pressures	centimetres water	cm H_2O
	millimetres mercury	mm Hg
Volume	litres	L
Time	seconds	sec
	minutes	min
	hours	hr
Volume per unit time	Litres per minute	L/min

SYMBOLS

P	pressure
p	partial pressure
V	volume of gas
f	frequency
Gd	Gadolinium
Sn	Tin
Ce	Cerium
Ru	Ruthenium
pa	pulmonary artery
ra	right atrium

ABBREVIATIONS

BP	systemic arterial blood pressure
CO	cardiac output
CO_2	carbon dioxide
FEV1	forced expiratory volume in one second
FVC	forced vital capacity
LDF	laser Doppler flowmetry
O_2	oxygen
PEEP	Positive end-expiratory pressure
Ti	inspired temperature
Tb	body temperature

CHAPTER 1

INTRODUCTION, ANATOMY AND PHYSIOLOGY OF THE AIRWAY VASCULATURE

1.1 INTRODUCTION

The lung is a unique structure in that it has two sources of blood supply, one from the systemic circulation and one from the pulmonary circulation (Aviado, 1965). The systemic blood supply, originally observed by Galen (A.D. 130-201) and drawn by Leonardo da Vinci (discussed in Cudkowicz, 1952), is relatively small in volume, receiving only 1-2% of the cardiac output (Cudkowicz, 1979), compared to the pulmonary circulation, which receives the entire output of the right ventricle. However the systemic circulation is important, as it is the major source of blood supply to the central airways, and also provides a source of blood to the lung parenchyma which may become crucially important when the pulmonary circulation is occluded (Virchow, 1847), or when pathological lesions such as tumours (Wood & Miller, 1938), or localised infections (Liebow et al, 1949), are present. Recently, interest in the systemic blood supply to the lungs has increased with the realisation that vascular responses in the central airways may be important in the pathogenesis of exercise induced asthma (Gilbert et al, 1987), and that the airway mucosal circulation may be important in the distribution of systemically administered drugs to the airways and in the absorption of inhaled drugs and toxins (Kelly et al, 1986).

Present knowledge of the systemic blood supply to the lungs is largely derived from animal studies (Deffebach et al, 1986), since methods of measuring this blood supply have been invasive, and have not therefore been applicable to human subjects. Information regarding the blood supply to the airways in particular is even more limited, since many previous studies have been concerned with total systemic blood supply to the lungs, including the component distributed to the lung parenchyma. It would clearly be desirable to have a non-invasive

technique for measuring airway wall blood flow, particularly to examine the role of the airway circulation in the pathogenesis of airway disease and in drug absorption from, and drug delivery to, the lung. Laser Doppler flowmetry (LDF) is a non-invasive method of measuring microcirculatory blood flow which has been widely applied to study blood flow to skin, nasal mucsosa, and gut wall. For studies of gastric and intestinal blood flow, the LDF probe has been positioned using fibreoptic endoscopy, permitting studies in intact animals and humans as discussed later. The objective of this study was to examine whether laser Doppler flowmetry could be similarly applied during bronchoscopy in both animals and humans to measure blood flow to the central airways. Results using the LDF technique were compared with other established techniques.

The studies were performed in two animal species, sheep and dogs, and in human volunteer subjects. In the animal studies, responses of the central airway blood flow to acute and sustained changes in systemic perfusion pressure and airway pressure were measured, and signals obtained using laser Doppler flowmetry were compared with two established invasive techniques, an isolated perfused bronchial artery preparation (Wagner et al, 1987), and the reference flow technique using radiolabelled microspheres (Baile et al, 1982). The response of the airway circulation to hyperventilation of warm dry air was also studied in dogs, using both laser Doppler flowmetry and the reference flow technique. In the human studies, laser Doppler flow signals were obtained in the central airways of patients undergoing cardiopulmonary bypass for cardiac surgery, and in a small group of normal and asthmatic volunteers during fibreoptic bronchoscopy. These studies were designed to examine the feasibility of obtaining measurements, and to identify sources of variation and noise in the LDF signals. A

preliminary study of the effects of hyperventilation of cold dry and warm humid air on LDF signals in the trachea was also performed in the latter subjects.

In the remainder of this chapter, current knowledge of the anatomy and physiology of the central airway vasculature will be reviewed. The theory and previous applications of laser Doppler flowmetry will be discussed in chapter 2.

1.2 ANATOMY AND PHYSIOLOGY OF THE CENTRAL AIRWAY VASCULATURE

The blood supply to the central airways is derived principally from branches of the thyroid arteries and the bronchial arteries. Since species variation occurs, canine, sheep, and human anatomy will be described separately.

1.2.1 Canine Anatomy

Arterial blood supply to the trachea of the dog is derived from the carotid arterial system via the cranial thyroid artery and the right and left caudal thyroid arteries in its upper two-thirds, and via branches of the bronchial arteries in its lower one-third (Miller et al, 1964). The major bronchi are supplied by the bronchial arteries whose origins are variable. Notkovich (1957) found the commonest pattern to be one right and one left bronchial artery, though variation in number of vessels did occur. In all cases, however, the origin of the vessels was from the intercostal arteries, and never from the aorta. This pattern was also seen by Ellis et al (1951). Other authors, however, have described origin from the internal mammary and pericardial arteries (Berry et al, 1931). Anastomoses with the oesophageal, subclavian, and mediastinal vessels occur (Horine & Warner, 1932).

The systemic vessels course along the outer surface of the central airways, giving rise to perforating vessels which enter between the cartilage rings. These vessels in turn divide into an anterior and a posterior branch, from which branches extend to supply a submucosal plexus (Sobin et al, 1963). Laitinen et al (1986a) have shown that, in both the trachea and bronchi of dogs, there is a capillary plexus close to the epithelial basement membrane, with connections to a deeper mucosal plexus of larger venules and

arterioles. From this plexus, larger venules then return along the course of the perforating arteries and arterioles.

Venous drainage from the tracheal and bronchial circulation may be to the right or left atrium. In general, drainage from the extrapulmonary airways is to the right atrium, and from the intrapulmonary airways is to the left atrium via the pulmonary veins. The major parahilar bronchial veins drain into the azygos vein on the right side, and the azygos, intercostal, or oesophageal veins on the left side (Berry et al, 1931), and the anterior bronchial veins drain to the internal mammary veins. Anastomoses of the bronchial system with the pulmonary system have been described at arterial level (Parker et al, 1957), and at capillary and venous levels (Berry et al, 1931). Since the bronchial arterial system has such rich anastomoses with the pulmonary circulation, it is at least theoretically possible that retrograde filling from the pulmonary circulation may occur. This has been demonstrated experimentally (Barman et al, 1988), and may occur pathologically in situations where the pulmonary arterial pressure is very high, e.g. mitral stenosis.

1.2.2 Sheep Anatomy

The systemic blood supply to the sheep lung is also derived from several sources. The cervical trachea is supplied by multiple small vessels arising from the common carotid arteries, whilst the segment of the trachea lying between the cervical region and the origin of the tracheal bronchus is supplied from the ramus bronchalis trachealis (tracheal bronchial vessel), which arises from the brachiocephalic trunk (Charan et al, 1987). This latter vessel may also supply the trachea between the origin of the tracheal bronchus and the main

carina, but in the majority of cases, another vessel, the ramus trachealis thoracica (thoracic tracheal vessel), arising either from the bronchoesophageal artery or directly from the aorta, supplies this region. The majority of the systemic blood supply to the remainder of the lungs is derived from the ramus bronchalis communis (common bronchial artery), a branch of the A. bronchoesophagea (bronchoesophageal artery). This vessel, previously termed the carinal artery, was thought in earlier studies to provide as much as 88% of the systemic supply to the sheep lung (Magno & Fishman, 1982), but subsequent studies suggest that its contribution is less, of the order of 50-70% at normal perfusion pressures (Charan et al, 1987, Gunther et al, 1987). There are other potential sources of systemic blood flow to the sheep lung. The oesophageal branch of the bronchoesophageal trunk gives off small branches which supply the visceral pleura, and these anastomose with the intrapulmonary bronchial vessels (Magno & Fishman, 1982, Charan et al, 1987). These are, however, thought not to contribute significantly to blood flow during normal perfusion pressures.

The microscopic arrangement of the airway wall blood flow is similar in sheep to that in dogs. The systemic vessels form an outer peribronchial plexus, and perforating vessels arising from this plexus traverse the muscularis to supply an inner submucosa plexus of vessels of 5-20 microns in diameter (Charan et al, 1984). Recently, however, an additional plexus of larger diameter sinuses (50 - 500 microns in diameter) has been described in the trachea (Hill et al, 1989). These vessels are thought to communicate with arterioles and venules, and lie mainly adjacent to cartilage plates. Their function is not yet known.

The venous drainage of the extrapulmonary bronchi is by way of

the bronchial vein which drains, in turn, into the left azygos vein (Charan et al, 1984), and by other small veins which join to from a mediastinal vein draining to the superior vena cava (Charan et al, 1987). The intrapulmonary bronchial vessels, on the other hand, drain into the pulmonary vessels via anastomoses at the precapillary level (Charan et al, 1984, 1985).

1.2.3 Human Anatomy

In humans, blood supply to the upper portion of the trachea is derived from the inferior thyroid arteries (Miura & Grillo, 1966), whilst close to the main carina, anastomoses exist with branches of the bronchial arteries (Salassa et al, 1977). Blood supply to the major bronchi is by the bronchial arteries.

The origin of the bronchial arteries is variable. Miller (1925) described the usual arrangement as being one right bronchial artery and two left bronchial arteries. Other patterns including one right and one left, 2 right and one left, and even 2 right and 2 left were seen by Cauldwell et al (1948) and Liebow (1965), and indeed the last pattern may be the commonest. The right bronchial arteries arise from the upper intercostal arteries (Cauldwell et al, 1948), whilst the left bronchial arteries usually arise from the ventral surface of the aorta (Miller, 1925, Cauldwell et al, 1948, Pump, 1963, Liebow, 1965). A right bronchial artery usually supplies the posterior (membraneous) part of the trachea in its lower part, and the bronchial arteries then course along the posterior aspect of the bronchi (Cudkowicz & Armstrong, 1951). In addition to supplying the airways and the lung substance, they also give off branches to the oesophagus, pericardium, mediastinal lymph nodes, and coronary vessels (Cauldwell et al, 1948, Liebow, 1965).

The vessels are again arranged in an outer plexus of arterioles with branches passing through the muscular layer to provide a rich submucosal plexus (Cudkowicz & Armstrong, 1951, Miura & Grillo, 1966).

Venous drainage from the central airways is by way of the bronchial veins, draining to the azygos, hemiazygos, or intercostal veins (Miller, 1947, Liebow, 1953), and thence to the right atrium. In addition, the bronchial circulation has extensive anastomoses with the pulmonary circulation, certainly at the capillary and venular levels (Tobin, 1952, Von Hayek, 1960, Wagenvooort & Wagenvoort, 1967), and, more debatably, at the arteriolar level (Tobin, 1952). Marchand et al (1950) also described "true bronchial veins", which communicated directly with the pulmonary vein and left atrium, but the existence of these has not been confirmed by others.

The complexity of the systemic blood supply to the lungs, with its multiple origins and outflow pathways, combined with variation between species and between individuals within a single species, has led to the development of a variety of techniques to measure systemic blood flow. Each of these techniques has limitations, and in the next section, the best established techniques will be reviewed.

1.3 SYSTEMIC BLOOD SUPPLY TO THE LUNGS - METHODS OF MEASUREMENT

All previously described methods of studying the systemic blood flow to the lungs have been invasive and therefore largely confined to animal studies. Several approaches have been used. Major systemic vessels to the lungs have been isolated with or without the portion of the aorta from which they arise (Horine & Warner, 1932, Bruner & Schmidt, 1947, Horisberger & Rodbard, 1960, Murao, 1965, De Kock et al, 1966, Lung et al, 1976, Laitinen et al, 1986). The vessels have then been cannulated allowing manipulation of flow or pressure (De Kock et al, 1966), or measurements of flow have been made using electromagnetic (Bruner & Schmidt, 1947, Lung et al, 1976) or ultrasonic flow probes (Villablanca et al, 1987). In dogs, this method has proved suitable for measuring tracheal blood flow (De Kock et al, 1966), but it is not suitable for studying bronchial blood flow due to the variable origins of the bronchial arteries. In sheep, since a large proportion of the bronchial blood flow is derived from a single vessel (Magno & Fishman, 1982, Charan et al, 1987), this method has been successfully used (Wagner et al, 1987).

An alternative approach, also applied to sheep, has been to cannulate the bronchial artery and inject radiopaque dye. By use of a videodilution technique, blood flow in the bronchial artery can be derived relative to the total cardiac output. Actual blood flow can then be determined by simultaneous measurement of the cardiac output (Link et al, 1985, Parsons et al, 1985). The potential of this technique for human studies is limited by the radiation exposure necessary, and by the technical difficulty of cannulating normal human bronchial arteries.

Another method of measuring systemic blood flow to the lungs in animals is the reference flow technique using radioactive microspheres

(Malik & Tracy, 1980, Baile et al, 1982, Wu et al, 1988). The principle of this technique is as follows. Radiolabelled microspheres are injected into the left atrium or ventricle, and a reference blood flow sample is withdrawn from a systemic artery at a known flow rate during the injection of the microspheres. The microspheres are subsequently trapped in the microvasculature of all tissues, and the systemic blood flow to each tissue can be subsequently derived by sectioning the tissue, counting the radioactivity in the tissue sections and in the reference flow blood sample, and applying a simple proportion relating radioactive counts to flow rate (Baile et al, 1982). Up to four separate isotopes can be used, allowing blood flow measurements at four distinct time points in any one study. Using this technique, both total bronchial blood flow and the bronchopulmonary anastomotic component of bronchial blood flow can be measured. Total tracheal blood flow can also be measured and the proportion of tracheal blood flow supplying the mucosa can be determined by stripping off the mucosa from the remainder of the wall before counting the radioactivity (Baile et al, 1987a). The major limitations of this technique are that the animal must be killed to determine the tissue radioactivity, and that only four separate flow measurements can be obtained in any study.

A number of other techniques have been used to measure bronchopulmonary anastomotic flow in animals and humans. These include simultaneous determination of right and left ventricular outputs, the anastomotic flow being derived by subtraction (Cudkowicz et al, 1959a & 1959b), and measurements of bronchial flow and left ventricular vent flow when the pulmonary circulation is interrupted during cardiopulmonary bypass (State et al, 1957, Aramendia et al, 1962, Baile et al, 1985). These techniques do not include the portion

of the systemic blood flow to the central airways, which is the major area of interest in the present study.

Two recent animal models have provided further information on tracheal perfusion. Laitinen et al (1986) have used a dog model in which the superior thyroid artery is cannulated and perfused at a known flow rate while pressure is measured, allowing vascular resistance to be determined. In addition, these authors also measured mucosal thickness using a micromanipulator and probe system, allowing the effects of neurological and pharmacological stimuli on both vascular resistance and mucosal thickness to be studied. This technique requires extensive surgical preparation. Wanner et al (1988) have applied an inert soluble gas method to measure mucosal perfusion and water volume in an isolated segment of trachea. In this method, the uptake of an inert soluble gas, dimethyl ether, from a segment of airway isolated by inflating cuffs at a separation of approximately 15 cm, is determined by measuring the rate of flow of air into the segment which is necessary to replace the gas as it is taken up. Since the rate of uptake is dependent on the tissue water volume (transient state), and on the blood flow rate (steady state), both parameters can be determined. Potential difficulties in the use of this technique however include the effects on gas diffusion of varying volumes of intraluminal secretions leading to changes in transit time for the gas, and the difficulty of estimating the depth to which the gas penetrates and thus the portion of the airway wall blood flow which is being measured.

Irrespective of which technique is used to measure total systemic blood flow to the lungs or the component to the airways only, a number of physiological conditions and pharmacological agents are known to influence blood flow. It is thus important that these factors should

be controlled if the effect of a particular intervention on airway blood flow is being assessed, and these factors are therefore discussed in the following section.

1.4 DETERMINANTS OF SYSTEMIC BLOOD FLOW TO THE LUNGS

Previous studies have examined physiological and pharmacological determinants of systemic blood flow to the lungs. Most of the information is derived from animal studies, and, owing to the nature of the methods of measurement, the tracheal and bronchial arterial circulations have generally been described separately.

The major physiological determinants of blood flow include the systemic blood pressure, the left atrial pressure, the airway pressure, and, for the extrapulmonary airways, the right atrial pressure. Arterial and alveolar partial pressures of oxygen and carbon dioxide also influence blood flow.

1.4.1 Vascular and Airway Pressures

Bronchial and tracheal blood flow vary directly with systemic blood pressure (Williams & Towbin, 1955, Lung et al, 1976). Elevation of pulmonary arterial pressure (Auld et al, 1960, Charan et al, 1985), pulmonary venous pressure (Auld et al, 1960, Goetz et al, 1965), left atrial pressure (Goetz et al, 1965, Wagner & Mitzner, 1988), and right atrial pressure (Salisbury et al, 1957), may each reduce the total bronchial arterial blood flow since each may act as a downstream pressure at different sites within the bronchial circulation. The effects of airway pressure on systemic blood flow are more complex, and will be discussed in chapter 4, but, in summary, increases in airway pressure result in a rise in vascular resistance and in downstream vascular pressure, resulting in a reduction in blood flow

to the airways (Bruner & Schmidt, 1947, Baile et al, 1984, Cassidy et al, 1986, Wagner et al, 1987).

1.4.2 Gas Tensions

Conflicting evidence exists concerning the role of gas tensions in the modulation of systemic blood flow to the lungs, and differences have been observed between the reponse of the tracheal and the bronchial circulations. Sahin et al (1987) found that systemic hypoxia caused an increase in tracheal vascular resistance in dogs, mainly by action on the central nervous system, and this was consistent with previous observations (Baile et al, 1983). In contrast, in several studies, systemic arterial hypoxia increased bronchial arterial blood flow (Bruner & Schmidt, 1947, Charan et al, 1986, Wagner et al, 1988). This effect may be mediated via the carotid body (Alsberge et al, 1988). Alveolar hypoxia, however has been shown in some studies to increase blood flow (Lilker & Nagy, 1975 Wagner & Mitzner, 1988), and, in other studies, to decrease blood flow (Baile et al, 1983). This latter response may be due in part to increases in pulmonary arterial pressure associated with hypoxia. Wagner & Mitzner (1988) have suggested that a biphasic reponse may occur, with an initial vasodilation being followed by vasoconstriction. Systemic arterial hypercarbia also apparently increases tracheal vascular resistance (Sahin et al, 1987) but reduces bronchial vascular resistance (Bruner & Schmidt, 1947, Salisbury et al, 1957, Baile et al, 1983, Charan et al, 1986), while alveolar hypercarbia is apparently without effect (Jindal et al, 1984). In the case of both responses, it appears that local tissue effects of hypoxia and hypercarbia may differ from the effects mediated through the central nervous system (Sahin et al, 1987).

1.4.3 Neural Influences

The innervation of the tracheobronchial tree, in common with other systems, has cholinergic, adrenergic and peptidergic components (Barnes, 1986). Knowledge of the role of these components in relation to tracheobronchial circulation is derived from histological and histochemical studies, and from physiological studies examining the influence of nerve stimulation and administration of neurotransmitter and mediator substances on the blood flow through the circulation. Tracheal and bronchial circulations have often been studied separately, and species and methodological differences have led in some cases to apparently contradictory conclusions by different workers.

Innervation of the tracheobronchial vasculature

The distribution of nerve fibres in the airways has been examined using histochemical and immunocytochemical methods. Early studies (Spencer & Loef, 1964) identified nerves supplying bronchial vessels, and more recent studies have suggested that the majority of these are adrenergic or peptidergic nerves.

Cholinergic nerves are seen in smooth muscle and bronchial glands (Sheppard et al, 1983, Laitinen et al, 1985a), but have not been clearly identified in close relation to tracheobronchial vessels. The results of physiological studies however suggest that cholinergic receptors are present within the tracheobronchial vasculature.

In contrast, adrenergic nerves also supply bronchial smooth muscle and glands (Partanen et al, 1982, Pack et al, 1984, Laitinen et al, 1985) but are, in addition, found in close association with the bronchial vessels (Partanen et al, 1982, Doidge et al, 1982, Sheppard

et al, 1983). Alpha-1, beta-1, and beta-2 receptors are present on bronchial arterial smooth muscle strips (Arowolo et al, 1980), and beta receptor density appears greater on bronchial arteries than on femoral arteries (Lung et al, 1976).

Peptidergic nerves, of both motor and sensory types, are present in the airways of various species (Barnes, 1984), and evidence is growing to suggest that they may be of considerable importance in the control of the tracheobronchial vasculature. In many cases, nerve fibres contain more than one neuropeptide transmitter, allowing the potential for co-transmission. Vasoactive intestinal polypeptide (VIP), occurs in parasympathetic motor nerves related to the bronchial vessels (Dey et al, 1981, Laitinen et al, 1985b, Uddman & Sundler, 1986), and two other related peptides, peptide histidine isoleucine (PHI) and peptide histidine methionine (PHM), may coexist with VIP (Itoh et al, 1983, Lundberg et al, 1984). Substance-P immunoreactive sensory nerves have also been identified in relation to tracheal and bronchial blood vessels, and calcitonin gene-related peptide may coexist with substance P (Uddman and Sundler, 1986), as may neurokinin A and B. Neuropeptide Y-immunoreactive nerve cell bodies are found in sympathetic ganglia, and NPY may coexist with noradrenaline in sympathetic nerve cell bodies and fibres (Uddman and Sundler, 1986).

The results of physiological studies tend to support the concept that the adrenergic and peptidergic systems are the important neural regulators of tracheobronchial blood flow. In particular, tachykinins, peptides found in afferent nerve endings within the lung (Lundberg et al, 1984), may have an important role in regulating airway blood flow.

Cholinergic system

Although cholinergic innervation of the airways is important in the control of airway calibre and mucus gland function (Olsen et al, 1965, Vincent et al, 1970, Davis et al, 1976, Baker et al, 1985), its role in the control of the vasculature is less clear. Anatomic evidence of cholinergic innervation of the tracheobronchial vasculature is inconclusive, and the results of physiological studies are contradictory. Although some workers have demonstrated a fall in bronchial blood flow (Baile et al, 1986) or a rise in bronchial vascular resistance after vagotomy (Horisberger & Rodbard, 1960), this response has not been confirmed by others (Martinez deLetona et al, 1961, Jindal et al, 1985b). The significance of these results in relation to cholinergic innervation is difficult to interpret in view of the systemic haemodynamic effects of vagotomy, and the possible involvement of non-cholinergic transmission (see below). An increase in bronchial blood flow upon vagal nerve stimulation is a consistent observation (Bruner & Schmidt, 1947, deLetona et al, 1961, Murao, 1965, Jindal et al, 1985b), suggesting cholinergic control. However, in a recent study in pigs, Matran et al (1989a) have suggested that this response is exclusively regulated by the sensory peptidergic component of the vagus. Carotid body stimulation by perfusion with hypoxic blood has also been shown to increase bronchial blood flow in sheep, a response which is partially blocked by atropine, suggesting a cholinergic component (Alsberge et al, 1988).

With respect to the tracheal circulation, sectioning of either the superior laryngeal nerve (Laitinen et al, 1987b), or of the vagus (Sahin et al, 1987), does not affect baseline tracheal vascular resistance, suggesting that there is no significant resting cholinergic tone. As is the case with vagal stimulation and the

bronchial circulation, stimulation of the superior laryngeal nerve reduces tracheal vascular resistance, an effect which appears to have a cholinergic component (Laitinen et al, 1987b), although elements of non cholinergic transmission are equally important in this response (Laitinen et al, 1987b, Martling et al, 1987)(see below). In contrast to the bronchial circulation however, tracheal vascular resistance increases in response to hypoxic or chemical stimulation of the carotid body (Sahin et al, 1987). The reasons for this difference in response are not clear.

Responses to administration of cholinergic agonists, such as methacholine and acetylcholine are variable in magnitude, and may be dependent on route of administration. When administered intravascularly, these agents consistently cause an increase in bronchial blood flow (Martinez deLetona et al, 1961, deKock et al, 1966, Zapata-Ortiz et al, 1967, Link et al, 1985, Lakshminaryan et al, 1985), and an increase in tracheal blood flow (Himori & Taira, 1976) or a fall in tracheal vascular resistance (Laitinen et al, 1986b, 1987c). However, when given by aerosol inhalation, methacholine increases bronchial blood flow (Bruner & Schmidt, 1947, Parsons et al, 1985) but has no effect on tracheal mucosal blood flow measured by the inert soluble gas uptake method (Barker et al, 1988).

These studies therefore show that although there are cholinergic receptors in the vasculature, there is little, if any, role for cholinergic nerves in determining resting vascular tone. The reasons for the differences between the tracheal and bronchial vasculature in response to vagal motor stimulation are unknown. They may be related to differences in experimental protocols, differences in distribution of cholinergic receptors, or perhaps to the effects of cholinergic stimulation on the pulmonary circulation, which provides a proportion

of the downstream resistance for the bronchial circulation, but not for the tracheal circulation.

Adrenergic System

The degree of sympathetic innervation of the airways varies amongst species. Cats have dense sympathetic innervation (Silva & Ross, 1974), whilst humans have rather sparse innervation (Pack & Richardson, 1984). However, since the adrenergic nerves are in close relation to the blood vessels, it might be expected that the adrenergic system would have a major role in the control of the tracheobronchial vasculature, and this is supported by physiological studies.

Stimulation of the of sympathetic nerve trunks reduces flow in the bronchial vessels (Bruner & Schmidt, 1947), and in the tracheal vessels (Martling et al, 1987), and many studies have demonstrated that administration of the alpha-agonist, phenylephrine, reduces flow or increases vascular resistance in the bronchial circulation (Bruner & Schmidt, 1947, Martinez deLetona et al, 1961, Lung et al, 1976, Link et al, 1985, Parsons et al, 1985, Charan et al, 1985) and in the tracheal circulation (Laitinen et al, 1986b, 1987c). Administration of adrenaline or noradrenaline also reduces blood flow or increases vascular resistance in the bronchial (Horisberger & Rodbard, 1960, Link et al, 1985, Charan et al, 1985) and tracheal vasculature (Himori & Taira, 1976), an effect blocked by phentolamine, an alpha-receptor antagonist.

Isoprenaline, which has combined alpha and beta agonist effects produces vasodilation when given alone, an effect blocked by the beta-receptor antagonist, propranolol (Himori & Taira, 1976). Topical administration of alpha- and beta-agonists by aerosol has been shown

respectively to decrease or increase tracheal mucosal blood flow in a fashion consistent with their effects on total tracheal blood flow (Barker et al, 1988).

Whether baseline bronchial vascular tone is under sympathetic control is not clear. Baile et al (1986) observed no effect of alpha- or beta-blockade on baseline bronchial blood flow, suggesting that there was no resting adrenergic vasoconstrictor or vasodilator tone. In the study of Barker et al (1988), the administration of adrenergic and cholinergic blockers again had no effect on resting tracheal mucosal blood flow, suggesting that there was no significant adrenergic or cholinergic tone in the vasculature under resting conditions. Himori & Taira (1976) however found that administration of the beta-receptor antagonist, propranolol, resulted in vasodilatation of the tracheal vessels, suggesting some resting sympathetic tone. The response was transient, however, (0.5-1 min) and may have been related to the local anaesthetic action of propranolol. Interpretation of studies involving the use of adrenergic antagonists to assess resting tone is therefore difficult, and the effect on blood flow of sectioning the sympathetic nerves has not been examined to date.

In summary, anatomical and physiological studies suggest an important role for the adrenergic system in the control of tracheobronchial blood flow. Vasoconstriction is mediated by alpha-receptors, and vasodilatation by beta-receptors. It seems likely however that there is little, if any, adrenergic tone under resting conditions.

Peptidergic system

Recent studies suggest that the peptidergic nerves may provide the most important neural regulation of the tracheobronchial circulation. Martling et al (1985) have shown that, in cats, vagal stimulation induces an increase in tracheobronchial blood flow which is resistant to the administration of atropine, but is abolished by the administration of hexamethonium, a ganglion-blocker. In a further study (Martling et al, 1987), stimulation of the superior laryngeal nerve in cats also caused an atropine resistant increase in flow, and the effects of vagal stimulation were found to be stimulus depedendent. Although the effects of low threshold vagal stimulation could be blocked by atropine plus a ganglion-blocking agent, high threshold stimulation still caused an increase in blood flow even in the presence of both atropine pretreatment and ganglion blockade. The authors proposed that this latter increase was due to antidromic stimulation of afferent nerves, and this was supported by the observation that mechanical irritation or the administration of capsaicin also resulted in increased blood flow. Laitinen et al (1987b) have also shown that, in dogs, stimulation of the peripheral end of the superior laryngeal nerve reduces tracheal vascular resistance. This fall is partially attenuated after atropine, and further attenuated, but still significant, after hexamethonium. These authors also suggest that the remaining increase is related to antidromic stimulation of afferent nerves. Thus the peptidergic system may induce increases in blood flow either by postganglionic nerve transmission, with VIP or PHI/PHM as the neurotransmitter, or by axon reflex activation of sensory afferent fibres, for which tachykinins are the neurotransmitters.

In other studies (Laitinen et al, 1986b, 1987a), close arterial

injection of neuropeptides has been shown to induce changes in tracheobronchial blood flow or vascular resistance. Vasodilator peptides include Neurokinin A, VIP, CGRP, Substance P, and PHI, in descending order of potency (Salonen et al, 1988). The effect of CGRP is particularly long lasting (Laitinen et al, 1987a, Salonen et al, 1988). Potency however may relate to route of administration or may vary between species since, in a study in which neuropeptides were administered by systemic intravenous injection to pigs (Matran et al, 1989b), substance P, VIP, PHI, CGRP and capsaicin all caused vasodilatation in the tracheobronchial vasculature, and substance P was the most potent vasodilator. NKA, substance P, and CGRP also relax canine bronchial arterial smooth muscle in vitro (McCormack et al, 1988). Two neuropeptides, Bombesin and Neuropeptide Y, are vasoconstrictors, (Laitinen et al, 1987a, Salonen et al, 1988), with NPY being longer lasting. Since NPY fibres have been identified in close relation to tracheobronchial blood vessels (Uddman & Sandler, 1986), it is possible that NPY may be important in modulating the effects of sympathetic nerve stimulation and noradrenaline release.

In summary, there is evidence of peptidergic innervation of bronchial blood vessels, and physiological studies confirm that neuropeptides have effects on tracheobronchial vascular resistance. The physiological and pathological consequences of these observations remain to be defined.

1.4.4 OTHER MEDIATORS

A number of other mediators also have effects on tracheal and bronchial vascular resistance. The leukotrienes LTC4 and LTD4 have been shown to reduce bronchial blood flow in sheep (Long et al, 1986), whilst their effect in the dog is to cause a small reduction in

tracheal vascular resistance (Laitinen et al, 1987c). Histamine acts as a vasodilator of both tracheal and bronchial vasculature (Bruner & Schmidt, 1947, De Kock et al, 1966, Lung et al, 1976, Link et al, 1985, Laitinen et al, 1986b), the bronchial action probably being mediated by H2 receptors (Long et al, 1985), and the major changes occurring in airway mucosa/submucosa and in central airways of >1mm diameter (Kramer et al, 1988). The prostaglandins are also known to influence tracheal and bronchial blood flow, with both prostacyclin (Deffebach et al, 1986) and PGF2alpha (Lakshminaryan et al, 1985) acting as vasodilators, and the cyclooxygenase inhibitors, indomethacin and ibuprofen, acting as vasoconstrictors (Long et al, 1985, Jindal et al, 1985, Deffebach et al, 1986, Warren & Powell, 1986).

In summary, a wide variety of physical, neural and hormonal stmuli may serve to alter blood flow in the tracheal and bronchial vasculature. The role of these various stimuli in physiological and pathological situations requires elucidation, especially in humans, where studies are relatively few. Laser Doppler flowmetry may provide a non-invasive method of studying blood flow to the human airways, allowing these stimuli to be further investigated. The next chapter will describe the background to the method of laser Doppler flowmetry.

CHAPTER 2

LASER DOPPLER FLOWMETRY - PRINCIPLES AND METHOD

2.1 HISTORICAL ASPECTS

The Doppler principle was originally described by a German physician, Christian Doppler, in 1842 (quoted in Holloway, 1983), and states that "sound which is emitted by an object in motion will be shifted in frequency relative to a stationary observer in proportion to the velocity of that object". Although originally described for sound, the principle is true for all forms of energy in wave form. Stated mathematically:

$$\Delta f = f_1 - f_o$$

where Δf is the Doppler frequency shift, f_1 is the frequency of energy emitted from the source, and f_o is the observed frequency. Where the energy is emitted from, and returns to the observer, as is the case with laser Doppler flowmetry, frequency changes occur on the way to and from the object, doubling the observed frequency shift.

The optical maser or laser (LASER = Light Amplification by Stimulated Emission of Radiation), a source of coherent light of a single stable frequency, was developed simultaneously in 1958 by Gould and by Schawlow and Townes.

Measurement of microcirculatory blood flow by laser Doppler flowmetry is, however, a relatively new technique, having been described initially by Stern in 1975. Prior to this, Cummins et al (1964) had predicted that the rate of motion of macromolecules in solution could be detected by the backscattering of laser light, and Yeh and Cummins (1964) had used the technique to measure fluid flow in a 10 cm diameter tube. In 1972, Riva et al measured the flow of 0.56 micron diameter polystyrene beads and blood through 200 micron diameter capillary tubes, and found that the flow rate measured by a

laser Doppler system corresponded with a measured pump flow rate. In a further study, Riva et al (1979) measured blood flow in the retinal artery of a rabbit using their system, and although they had no gold standard by which to assess the accuracy of the method, their results corresponded with flow rates measured in similar sized vessels in dogs. In 1975, Tanaka and Benedek demonstrated that laser light could be carried through a fibreoptic catheter of 0.5mm diameter, and used their system to measure blood flow in the femoral vein of a rabbit. Other early applications of detection of the Doppler shift of laser light included measurement of velocity of red blood cells in the hamster cheek pouch by Einav et al (1975 a,b), measurement of flow in rabbit mesenteric vessels by Le-Cong and Zweifach (1979), measurement of ciliary activity of cells by Lee and Verdugo (1976), and measurement of bacterial motion by Nossal and Chen (1972). Assessment of microcirculatory blood flow in an intact system, the skin, using laser Doppler flowmetry was first described by Stern in 1975 and is discussed in more detail in the next section.

2.2 PRINCIPLES OF LASER DOPPLER FLOWMETRY

Measurement of microcirculatory blood flow by laser Doppler flowmetry is based on the the observation that when photons of light are shone into tissue they undergo multiple collisions with the cells, and a small proportion are eventually returned to the tissue surface, causing the tissue to glow. This is called backscattering of light. Photons which collide with stationary tissue are backscattered without any alteration in frequency, whereas those which collide with moving blood cells undergo a Doppler frequency shift. Any moving blood cell may cause a photon to undergo a Doppler shift, but since red cells are numerically the most important cells in the microvasculature, the

theory has been derived for red cells. In most tissues, photons striking red cells, and therefore undergoing a Doppler shift, are thought to constitute a small proportion (<0.1%) of the total backscattered light (Bonner and Nossal, 1981). The incident laser light is monochromatic, i.e. of a single, stable frequency, and is coherent, i.e. it has all of its waves in phase. When it is directed into a microvascular bed, the backscattered light, which can be collected by an optical receiving fibre, is comprised of both Doppler-shifted and non-shifted components. As light is of a very high frequency, direct measurement of the Doppler frequency shift cannot be made electronically. A photodetector is therefore used, in which the shifted and non-shifted components interact to produce an optical beat frequency. This process is known as a heterodyne interaction. In fact, many different beat frequencies are produced, a phenomenon called spectral broadening. This is because the red cells move at a variety of velocities in the microcirculatory bed, because photons are scattered at many small angles by the red cells, and because the light is also diffusely scattered by the static tissue matrix (Bonner & Nossal, 1981). The other consequence of this diffuse scattering is that the pathways of photons reaching erythrocytes within the tissue is totally random, and flow measurements derived by the technique are therefore non-directional.

The optical theory of laser Doppler flowmetry has been described by Bonner and Nossal (1981). As a photon of incident light strikes a moving red blood cell, it undergoes a small deflection from its original path. The mean angle of deflection or scattering angle is 5.4°. On average, the Doppler shift imparted to a photon by a moving red blood cell is 0.3 kHz/mm/sec, and the Doppler shift frequencies thus produced fall in the audio range of 30-20,000 Hz. By examining

the spectrum of frequencies produced, a term which is proportional to the velocity of red blood cells in the tissue can be determined.

In the early models of laser Doppler flowmeter, a signal processor which generated the first moment of the unnormalised power spectral density as a continuous output signal was used. The theoretical relationship developed by Bonner and Nossal to describe tissue blood flow measurement by laser Doppler flowmetry is of the form:

$$w = \frac{(V^2)\cdot^5 . \beta . f(\bar{m})}{12 . \varepsilon . a}$$

where w is the normalised first moment of the Doppler frequency spectrum, $(V^2)\cdot^5$ is the root mean square speed of moving red cells, a is the radius of an equivalent scatterer, ε is an empirical factor related to the shape of cells, β is an instrumental factor, depending on the optical coherence of the signal at detection, and $\bar{m}$ is the average number of collisions that a photon makes with moving red blood cells. When $(\bar{m})$ is less than 1, then the function, $f(\bar{m})$, is linear, i.e. w increases linearly with red cell flux. If, however, $\bar{m}$ is greater than 1, then each photon may undergo multiple Doppler shifts, and homodyne interactions, i.e. the different Doppler shifted signals interacting with each other rather than the unshifted reference beam, may become important. For values of $\bar{m}$ between 2 and 4, w varies with the square root of $\bar{m}$ (Bonner & Nossal, 1981). Theory suggests that values of $\bar{m}$ greater than 2 occur when the tissue blood volume is greater than 0.06 ml/g of tissue. The early instruments designed from this theory provided linear scaling at relatively low red cell velocities and concentrations, but were non-linear at higher velocities and concentrations (Bonner & Nossal, 1981, Nilsson, 1980b).

Newer signal processors were then developed, in which the amplitude, as well as the frequency, of the AC output was used to correct for the volume of red cells (Nilsson, 1984, Haumschild, 1986). This type of signal processing is incorporated in the instrument used in the present studies (Haumschild, 1986). In this instrument, all photons returning to the photodetector produce a DC offset voltage. Mixing of Doppler shifted and non-shifted signals produces a small AC signal, the amplitude of which is proportional to the number of photons undergoing a Doppler shift, i.e. the red cell volume, and the frequency of which is proportional to the velocity of the red cells (figure 2.1). The instrument incorporates a microprocessor by means of which the ratio of the AC to DC ratio is linearised and displayed as volume, and the mean frequency, derived from spectral analysis, is displayed as velocity. The product of these two values is displayed as blood flow, though should more correctly be defined as red cell flux. This latter point may be of signifcance in interpretation of the results of the present studies, as discussed later.

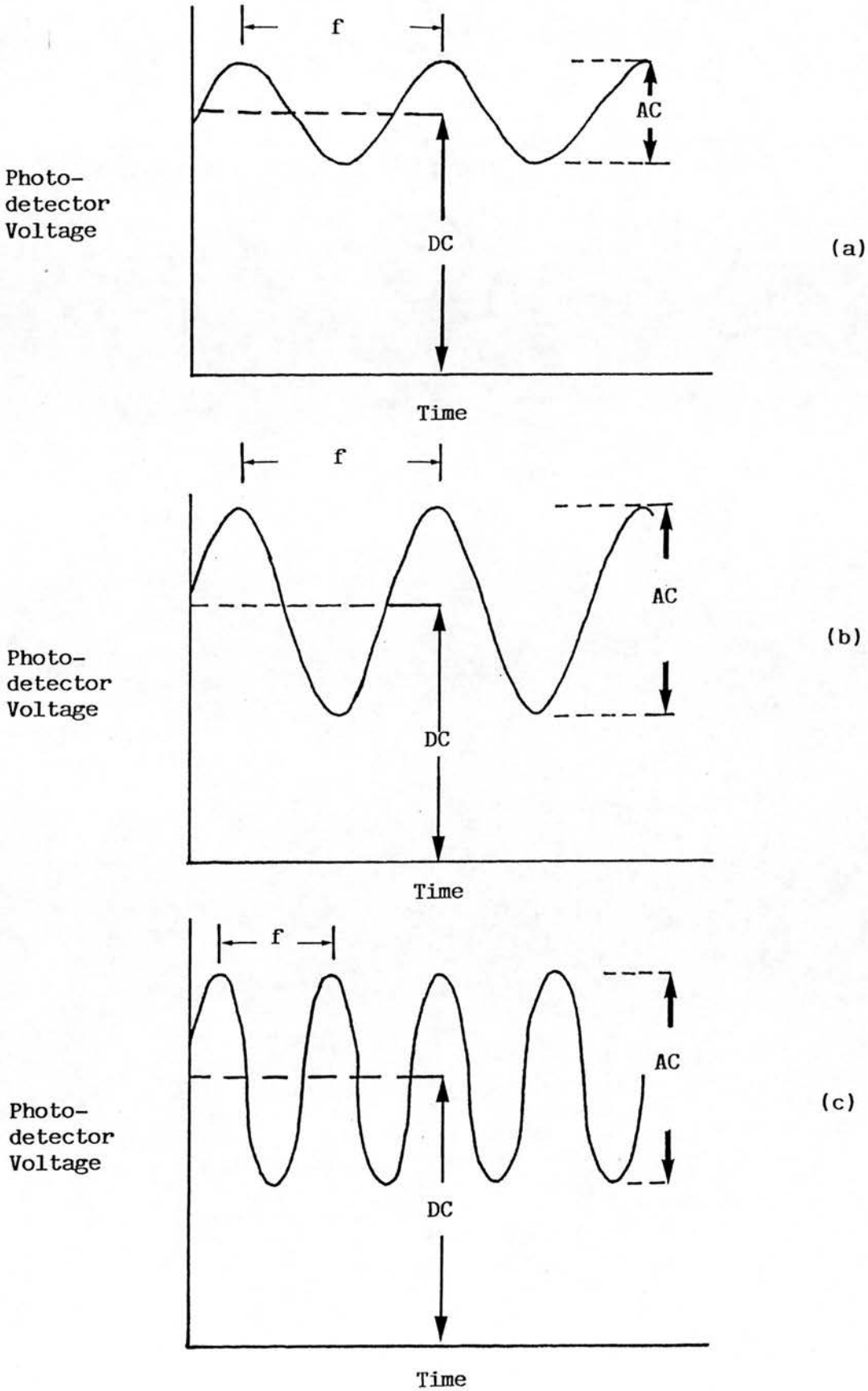


Figure 2.1. A single particle moving through a large volume of stationary tissue produces an offset voltage (DC) with a small AC component (a). Multiple particles moving at the same velocity increase the magnitude of only the AC component (b). Multiple particles moving at a higher velocity increase the frequency component (f) of the AC component (c).

2.3 DEVELOPMENT OF LASER DOPPLER FLOWMETERS

In Stern's original report (1975), a helium neon laser was used to illuminate skin in a fingertip. The backscattered light was detected by a photomultiplier tube, and the output from this, after filtering, was processed by a spectrum analyser. In a later report, Stern et al (1977) suggested that the bandwidth of the Doppler spectrum should scale in proportion to the red cell velocities, providing the geometry of the vascular bed did not change, and thus used the root-mean-square of the Doppler bandwidth as the flow parameter. This system correlated well for the measurement of skin blood flow with the ^{133}Xenon clearance method, and was also used to measure rat cortical and medullary renal blood flow (Stern et al, 1979), where it correlated with radioactive microsphere measurements and also with total renal arterial blood flow measured by an electromagnetic flow probe on the renal artery. The laser Doppler measurements did, however, appear to underestimate decreases in blood flow at low flows.

Other authors (Holloway and Watkins, 1977, Watkins and Holloway, 1978) devised a different system, also based on a helium-neon laser, but using a photodiode instead of a photomultiplier tube, and a fibreoptic system to carry light to and from the surface to be examined. This system was more easily applicable in the clinical setting due to its portability and ease of application of the flow probe, and this was demonstrated in its use to measure skin blood flow (Holloway, 1980).

Yet another bandwidth processing algorithm was used by Nilsson et al (1980a&b). In their instrument, several receiving fibres were used and were divided in 2 groups, each group carrying returning light to a separate photodiode. Laser noise, which was common to both groups of

fibres, was then rejected using a differential amplifier, while the Doppler signal, which was a random signal, was retained. Nilsson (1984) later further modified his instrument, developing a new signal processor which took into account multiple scattering of of photons by moving red cells, and was capable of monitoring higher blood flow rates.

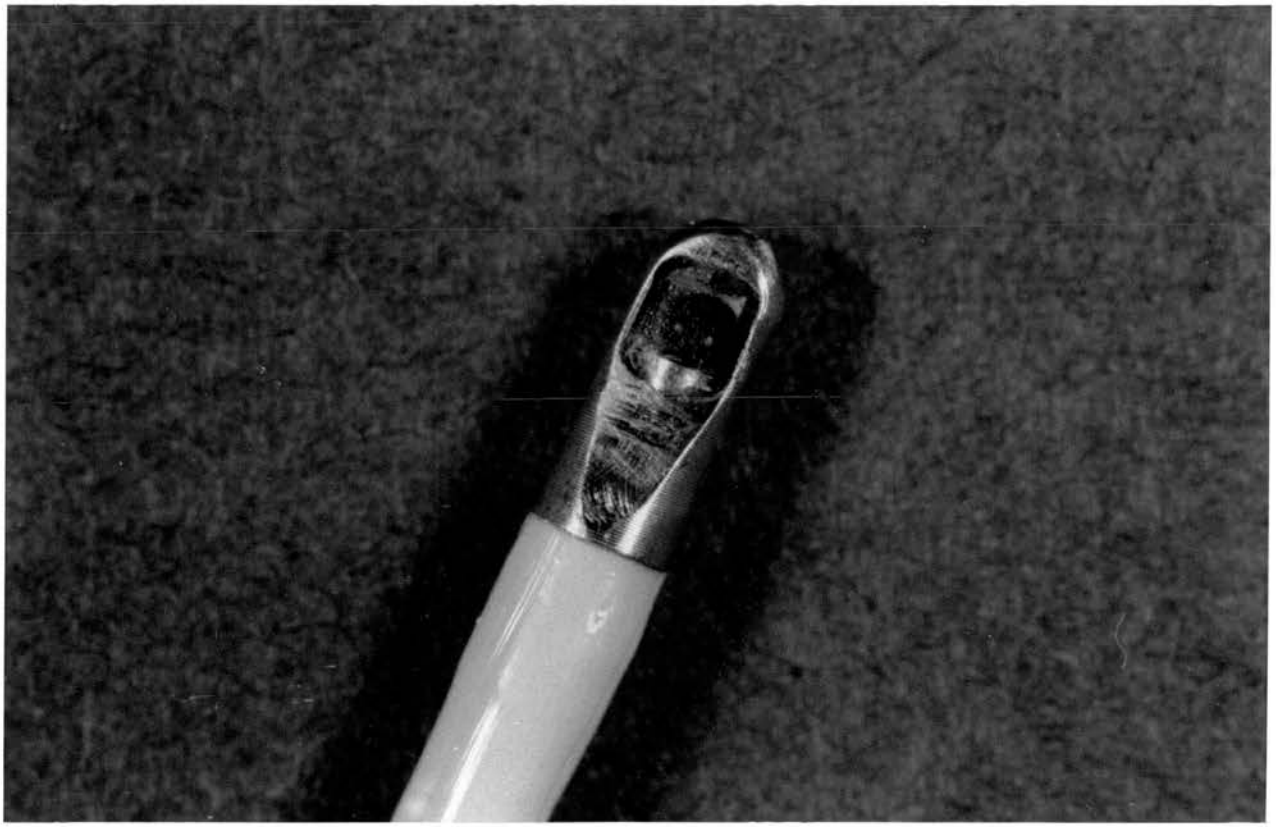
In current practice, two different types of laser are in use for the measurement of microcirculatory blood flow. The gas laser type (Helium-neon laser, operating wavelength 632.8nm) has been widely used for the measurement of blood flow in skin (Watkins & Holloway, 1978), nasal mucosa (Druce et al, 1984), renal cortex (Stern et al, 1979), testicle (Damber et al, 1986), and gastric (Kiel et al, 1985) and intestinal mucosa (Shepherd & Riedel, 1982). This system has certain optical advantages, producing a collimated output beam and a stable emission spectrum which is not temperature dependent (Haumschild, 1986). It is used in conjunction with an analogue signal processor. The major disadvantages with this system have been its large size and the need for a high voltage power supply for operation.

The instrument used in the present studies is a commercially available flowmeter (Laserflo BPM 403, TSI inc, St Paul, Mn) of different construction, having a laser diode source with an operating wavelength of 780-800nm. Potential optical disadvantages with this system include a divergent output beam, necessitating a focussing system, a short coherence length, and temperature dependence, i.e. small changes in ambient temperature may affect the wavelength of emitted light (Haumschild, 1986). Additional electronic circuitry is included to correct for this. This system uses a digital signal processing system, and has advantages over the helium-neon laser, in that it provides information related to both volume and velocity as

well as flow. The instrument carries both emitting and receiving fibres in a single fiberoptic cable, 2 metres in length, and 2mm in diameter. The fibres are separated by a distance of 0.5mm. The probe has a prismatic tip, which allows both transmitted and received light to be turned through an angle of 90°, such that it is at right angles to the long axis of the probe (figure 2.2). Volume, expressed as the number of Doppler shifts per photon, and velocity, expressed in Hz x 10^3, are measured in real time, and flow is continuously derived from these. The signals are displayed in digital form on the perfusion monitor, or can be output to an external recorder. The output rates are updated 10 times per second. The time over which flow, volume and velocity data are averaged can be selected at 0.1, 0.2, 0.5, 1, or 5 seconds, depending on whether the investigator wishes to study individual components of the perfusion waveform or mean levels of the perfusion waveform.

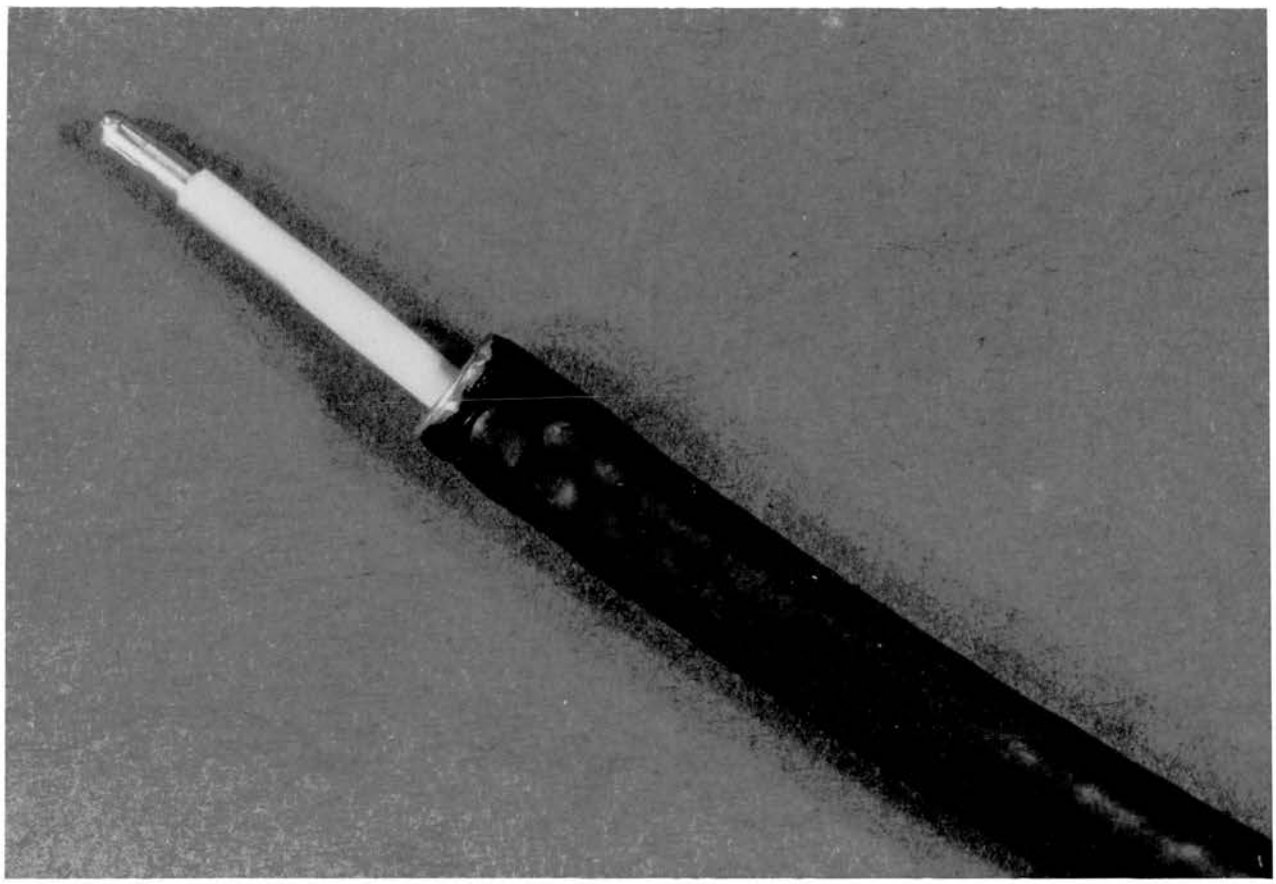
Both types of laser have a low optical output, and are classified by the United States Food and Drug Administration, Center for Devices and Radiological Health, as Class I lasers. This implies that they are completely safe for the operator and the subject or patient under study for unaided viewing.

fig 2.2 (a)



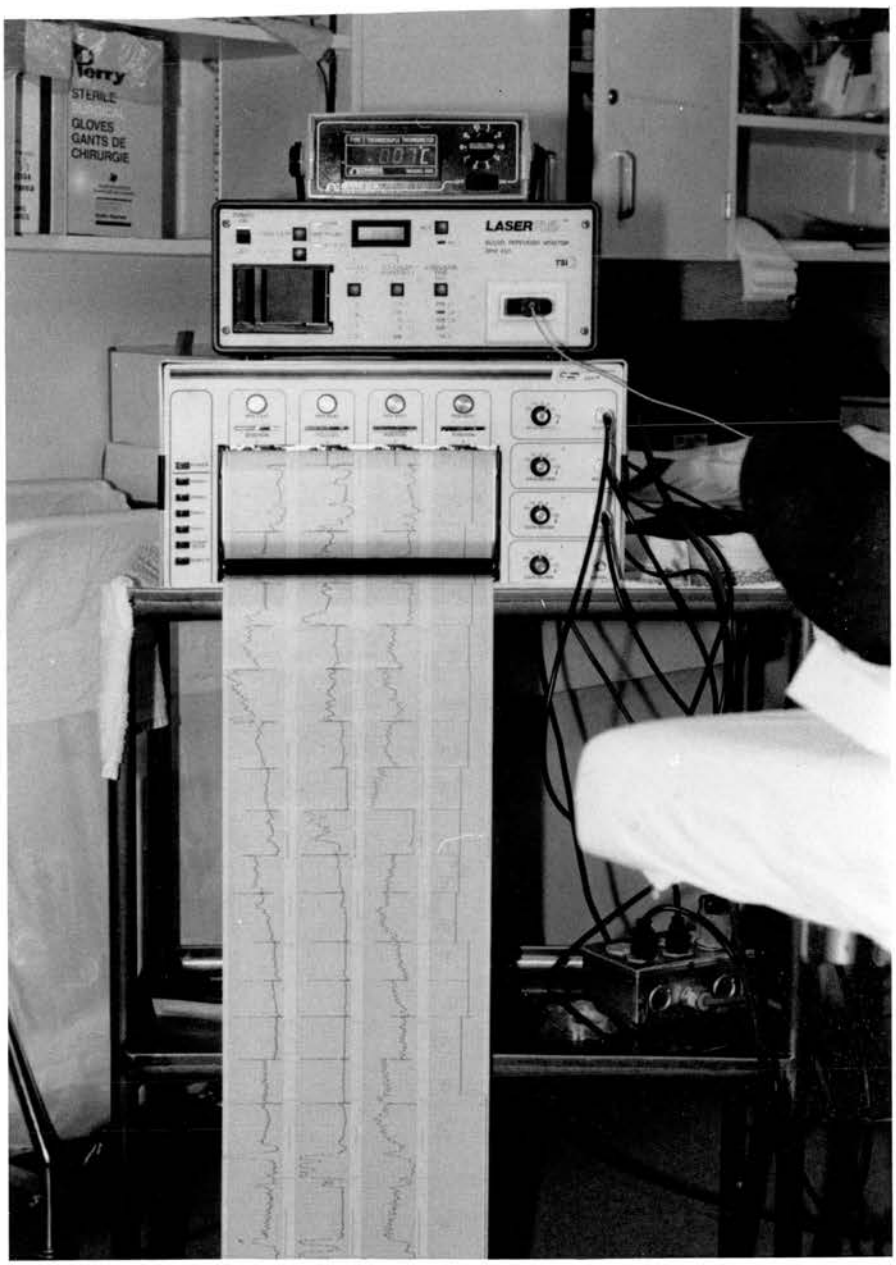
Close up view of the tip of the laser Doppler probe to show the prismatic tip which allows measurements to be made at right angles to the long axis of the probe

fig 2.2 (b)



The tip of the LDF probe extends 2-3 cm distal to the tip of the fibreoptic bronchoscope

fig 2.2 (c)



The LDF signal processor is shown, together with the 4-channel recorder displaying flow, volume and velocity signals

2.4 PREVIOUS APPLICATIONS OF LASER DOPPLER FLOWMETRY IN BIOLOGICAL SYSTEMS

Laser Doppler flowmetry has been used to measure blood flow in a variety of organs and species, and has been compared with other methods of measuring microcirculatory blood flow in a number of these studies. In this section, these studies will be reviewed, and their relationship to the present studies will be further discussed in chapter 8.

Organs studied using LDF include the kidney (Stern et al, 1977, 1979), testis (Damber et al, 1986), brain (Rosenblum et al, 1987) and skeletal muscle (Tahmoush et al, 1983, Tyml and Ellis, 1985). However, the systems in which LDF has been most widely studied are the gastrointestinal tract and skin, and these will be discussed in more detail since they have relevance to the study of airway blood flow.

Gastric blood flow has been studied by several authors. In a study of canine gastric blood flow, LDF was compared with total flow to an isolated gastric flap measured by an electromagnetic flowmeter (Kiel et al, 1985). Isoprenaline was given to prevent autoregulation of blood flow, and a linear correlation was observed between the techniques. Fluctuation in the LDF values was observed, which did not appear to relate to blood flow, but rather to electrical activity in the gastric muscularis. Following release of transient vascular occlusion, reactive hyperaemia was observed in the mucosa, but not in the total blood flow to the gut wall. Gana et al (1986) studied canine gastric blood flow in an isolated gastric flap, using LDF and the hydrogen gas clearance technique. They found that the techniques correlated over a range of blood flow from 20 to 180 ml/min/100g of tissue, but no single conversion factor could be determined to allow LDF flow to be quantitatively related to the hydrogen clearance value.

In each of the five dogs which they studied, they found different slopes for the regressions of LDF values against hydrogen clearance values. They also found that when the regression lines were extrapolated to zero, there was a positive intercept on the LDF axis. Holm-Rutili et al (1986) compared intravital microscopy and LDF in a rat gastric preparation and found that the blood flow response to pentagastrin infusion was similar with both techniques. Kvernebo et al (1986) studied human gastric blood flow using an LDF probe inserted through a gastroscope. They examined the effects of varying distance of the LDF probe from, and pressure by the probe on the gastric mucosa on the resultant LDF signal. They found that the signal remained stable when the probe was either held at 2-3 mm from the mucosa, or allowed to rest on the mucosa with slight or firm pressure. However if heavy pressure, defined as a loss of the visible halo around the probe head associated with bending of the probe, was applied, then the signal was reduced. They also observed that varying angulation of the probe relative to the mucosal surface from 0-60° from perpendicular had no effect on the signal. Artefacts due to peristalsis occurred but could be identified and the resultant signal discarded from analysis. There was considerable site-to-site variation in the LDF signals, the coefficient of variation between sites ranging from 33-39%. Chung et al (1988), using an identical LDF instrument to that employed in the present studies, also studied the effect of probe pressure on the signal, and actually measured application pressure using a spring loaded micromanipulator. They found that the ideal range of pressure to obtain satisfactory signals was from 5-20 gm/cm^2. In practical usage, this was equivalent to pressure producing mild dimpling of the mucosa. They also compared LDF with the H_2 clearance method in canine stomachs, and found that there was a positive

intercept for LDF when plots of LDF versus H_2 clearance measurements were extrapolated to zero. Ahn et al (1986c) also used an LDF probe inserted through the suction channel of a gastroscope to measure human gastric blood flow, and found lower values after vagotomy.

Intestinal blood flow has also been examined using LDF probes. Shepherd and Reidel (1982) compared LDF measurements with electromagnetic total flow measurements in an isolated loop of canine intestine. Correlation between the LDF and electromagnetic signals was good, but again a positive LDF intercept, i.e. LDF value at extrapolated zero flow, was observed. In further studies, they found that instillation of glucose and bile selectively increased intestinal mucosal blood flow (1985), and that autoregulatory escape was greater in the mucosa than in the muscularis (1988). Ahn and colleagues have also studied intestinal blood flow, using a different LDF instrument to that used by Shepherd and colleagues, and have reported a number of observations which differ from those of Shepherd et al. In one study (Ahn et al, 1986a) LDF was found to underestimate higher flows (> 40 ml/min/100g) in an isolated segment of human small intestine in which total segment flow was determined by venous collection. Interference with the LDF signals due to peristaltic waves and loss of optical coupling also posed problems in that study. In further studies, the same authors used an LDF instrument with a different signal processor and found linear responses up to 250 ml/min/100g in both cat intestine (1987) and human colon (1986b). In the latter study, they also demonstrated that that particular LDF instrument measured blood flow through the full thickness of the intestinal wall, and that the LDF measurements were related to the wall thickness. Since wall thickness varied between the sites at which LDF measurements were obtained, they corrected their LDF readings for the thickness of the gut wall

measured histologically at each site, and were thus able to further improve the correlation between LDF measurements and total venous outflow. Clearly this latter approach precludes measurements in an intact animal.

Many authors have examined skin blood flow using laser Doppler flow probes, and this literature will not be extensively reviewed. In one important study, however, Johnson et al (1984) compared total blood flow to the forearm of volunteers, measured by venous occlusion plethysmography (FBF), with LDF measurements at six sites on the skin of the forearm. They found a good correlation within each study between total blood flow and LDF measurements when blood flow was altered by changing the subject's temperature. However, there was marked site-to-site variation in LDF signals, the coefficient of variation between sites within subjects ranging from 23 to 75 % under resting blood flow conditions, and the slopes of the LDF versus FBF relationhips varied three-fold amongst studies. They attributed the site-to-site variability in LDF signals under resting flow conditions to the variation in capillary density which has been shown to be greater than six-fold between $1mm^3$ sites in human skin (Landis, 1938). Saumet et al (1986) also studied forearm blood flow, using venous occlusion plethysmography to measure total flow and LDF and heat thermal clearance to measure skin blood flow. Good correlations between the measurements were again found, but the LDF signal at zero forearm blood flow, induced by inflating a cuff to above systolic pressure was always positive.

Nasal blood flow has also been examined using LDF. Druce et al (1984) found marked variation in flow within subjects between days, though on any single occasion, measurements were quite stable. They also demonstrated reduction in blood flow when oxymetazoline and

phenylephrine were applied. Olsson et al (1985) showed that in the human nose, the LDF probe which they were using could be moved up to 3.5 mm away from the mucosa without significant attenuation of the signal, and that peripheral cold stimulus reduced nasal blood flow. In a subsequent study (1986), they observed cyclical changes in nasal blood flow which were abolished by stellate ganglion blockade.

Thus, in all of these systems, some common characteristics of LDF measurements are seen. A signal may be present when flow, as measured by other techniques, is zero. Good correlations with other techniques are often demonstrated for individual experiments but the slopes of the relationships between LDF and other measurements may vary from site to site, or from subject to subject, leading to difficulty in establishing a single calibration factor for LDF measurements in any of the systems. Site-to-site variation in signals is observed in most microvascular beds, possibly relating to variations in capillary density, and/or the occurrence of local autoregulation of flow or vascular steal phenomena. These observations will be further discussed later in the light of the present studies.

The instrument used in this thesis has been evaluated in some detail by Shepherd et al (1987). These authors performed studies to examine the reproducibility of measurements in 2 mechanical models and in 2 biological systems. In one mechanical system, a cuvette was perfused with polystyrene particles or with a suspension of canine red blood cells, and in another, a revolving fluorocarbon disk was used to generate Doppler shifts of the incident light. The biological systems were an isolated perfused rat liver preparation and an isolated canine gastric flap. In both mechanical models and in the biological systems the instrument had a linear response to increasing flow. However, in the liver and canine flap preparations, the slope of

the relationships between the LDF signals and the total flow to the organ varied from site to site, and varied with time when repeated measurements were made at individual sites. These findings are similar to those described above for other LDF systems, and again may be due to variations in microvascular haematocrit or to the fact that the very small tissue volumes in which the LDF system measures flow do not necessarily reflect flow in the whole organ. Another characteristic, which is also true of other laser Doppler flowmeter systems, was that a small LDF signal was observed even when flow was set to zero in the cuvette model. This signal may represent Brownian motion of cells, movement of tissue matrix, or may be due to detection of background noise from laboratory equipment, and will be further discussed in Chapter 3.

2.5 MEASUREMENT TECHNIQUE

In all the present studies the technique of obtaining laser Doppler flow measurements was similar. A fibreoptic bronchoscope (Olympus BF type IT10) was introduced into the airways. The laser Doppler probe was inserted through the suction channel and extended 2-3cm distal to the tip of the bronchoscope (fig 2.2b). Under direct vision, the tip of the probe was allowed to rest gently on the airway mucosa such that the incident light was at a right angle to the mucosa and a continuous recording of the output signal was obtained. In all studies, flow, volume and velocity signals were displayed on an external recorder (figure 2.2c), and an averaging time of 1 second was used.

Several technical factors were found to be important if satisfactory measurements were to be obtained, and sources of artifact identified will be discussed in the next chapter.

CHAPTER 3

SOURCES OF ARTIFACT IN LASER DOPPLER SIGNALS

3.1 INTRODUCTION

In the present and previous studies, several technical factors have been found to be important if satisfactory measurements of blood flow are to be obtained using laser Doppler flowmetry. These include adequate contact of the probe head with the mucosal surface under study, absence of relative movement between the probe head and the mucosal surface, satisfactory ambient light conditions, and factors relating to the tissue under study (pigmentation, blood volume, contractility). This chapter describes some sources of artifact encountered in the present studies of laser Doppler flowmetry to measure airway blood flow. These were mainly identified in the initial study in dogs, described in chapter 4, but were found to occur also in sheep and in humans. The relationship of these sources of artifact to those described by previous authors is discussed.

3.2 PROBE POSITIONING

In the present studies, the probe tip was held, under direct vision, in light contact with the mucosa at an angle as close as possible to 90° during each recording period. The pressure exerted by the probe tip on the mucosa was not directly measured, although the flow signals appeared to remain stable during the application of light pressure, sufficient to produce slight dimpling of the mucosal surface. Previous studies, on other mucosal surfaces, have suggested that optimal laser Doppler signals are obtained when the probe tip is positioned in this fashion. Kvernebo et al (1986), studying gastric mucosal blood flow measured through a gastroscope in human volunteers, examined the effect on the LDF signal of pressure from the probe tip on the mucosal surface. They found that gentle or medium pressure of the probe tip on the mucosa (defined by visibility of the red laser

light on the mucosal surface) had no effect on the LDF signal, but that heavy pressure (defined by bending of the LDF probe and absence of visible red laser light on the mucosal surface) did reduce the LDF signal. In another study (Chung et al, 1988), in which endoscopic measurement of canine gastric blood flow was examined, the pressure exerted by the probe tip on the gastric mucosal surface was directly measured, and optimal signals were obtained between the range of pressures from 5 to 20gm/cm^2. This range of pressure was found to be associated with minor dimpling of the mucosal surface, and this criterion was used in our study to indicate adequate contact.

In Chung's study, low application pressures (<5 g/cm^2) were associated with loss of optical coupling, identified by an erratic spike and wave pattern. In the present studies, minor degrees of loss of contact of the probe with the mucosa resulted in erratic fluctuations of the signals, particularly the volume signal (Figure 3.1).

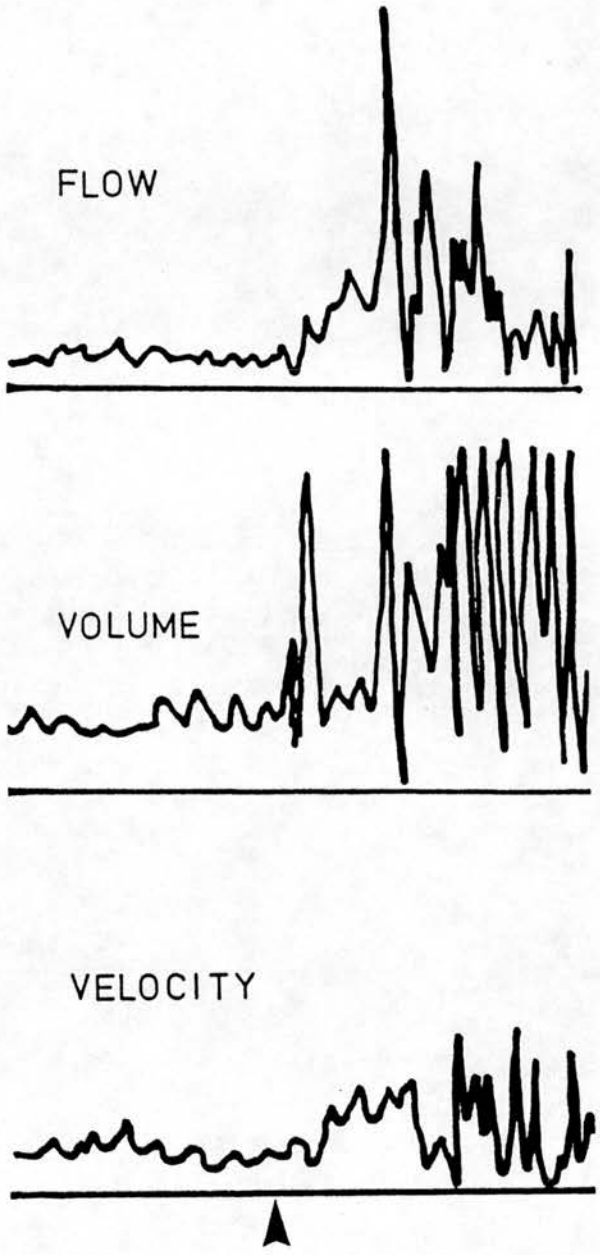


Figure 3.1. Loss of contact between the LDF probe and the mucosa is indicated by the arrow. Subsequent LDF signal shows erratic fluctuation in all signals, most pronounced in the volume signal.

If the loss of contact was of greater magnitude, sufficient to reduce the DC offset in the instrument, then an error code was displayed automatically by the instrument. In either of these circumstances, repositioning of the probe was necessary, and the signal related to loss of contact was rejected for the purposes of analysis. In the present studies, the major cause of this problem was the interposition of airway secretions between the probe tip and mucosa, and the frequency of episodes of loss of contact was much reduced when the animals were pretreated with atropine to reduce airway secretions. Erratic fluctuations in signals associated with loss of contact have also been observed by other authors using helium-neon laser Doppler flowmeters (Shepherd & Riedel, 1982, Ahn et al, 1986).

Angle of incidence of the laser light with the mucosal surface might also be expected to be important. However, since the direction of incident light is randomized by multiple scattering within the tissue matrix, and direction of flow is not being measured (see chapter 2), the angle of incidence of light directed onto the tissue surface is not critical. In the study of Kvernebo et al, the angle of the incident light was varied from 30–90° without alteration in the LDF signal. In the present studies, the design of the probe allowed the angle of incidence of the light to be held close to 90°, and not exceeding the range 60–90°, with relative ease.

3.3 MOTION ARTIFACTS

The major sources of motion artifact in studying tracheobronchial blood flow in intact animals and in man are ventilation and cardiac motion, which may result in motion of the probe relative to the mucosal surface under study.

When inserted through the suction channel of the fibreoptic

bronchoscope, the probe is held in constant relation to the bronchoscope. Ventilation, whether spontaneous or mechanical, results in movement of the mucosal surface relative to the bronchoscope and this is seen as large fluctuations in flow, volume and velocity signals (figure 3.2). This problem was overcome by temporarily stopping mechanical ventilation (figure 3.2), or in a spontaneously breathing, cooperative subject, by breath-holding.

Tissue motion due to transmitted cardiac impulse is a source of background noise in the laser Doppler signal, as will be discussed later. When excessive, cardiac motion may also render it difficult to maintain satisfactory contact of the probe with the mucosa. In the present studies, this was not a problem when the probe was in the trachea. However, when the probe was moved close to the main carina, and particularly into the left main bronchus, then this motion artifact became more significant. By allowing the LDF probe tip to extend beyond the tip of the bronchoscope by 2-3 cm, there was some potential for the probe to oscillate with the tissue whilst maintaining a constant relation to the mucosa. However, at some sites within the major bronchi, stable LDF measurements could not be obtained, and repositioning of the probe was necessary.

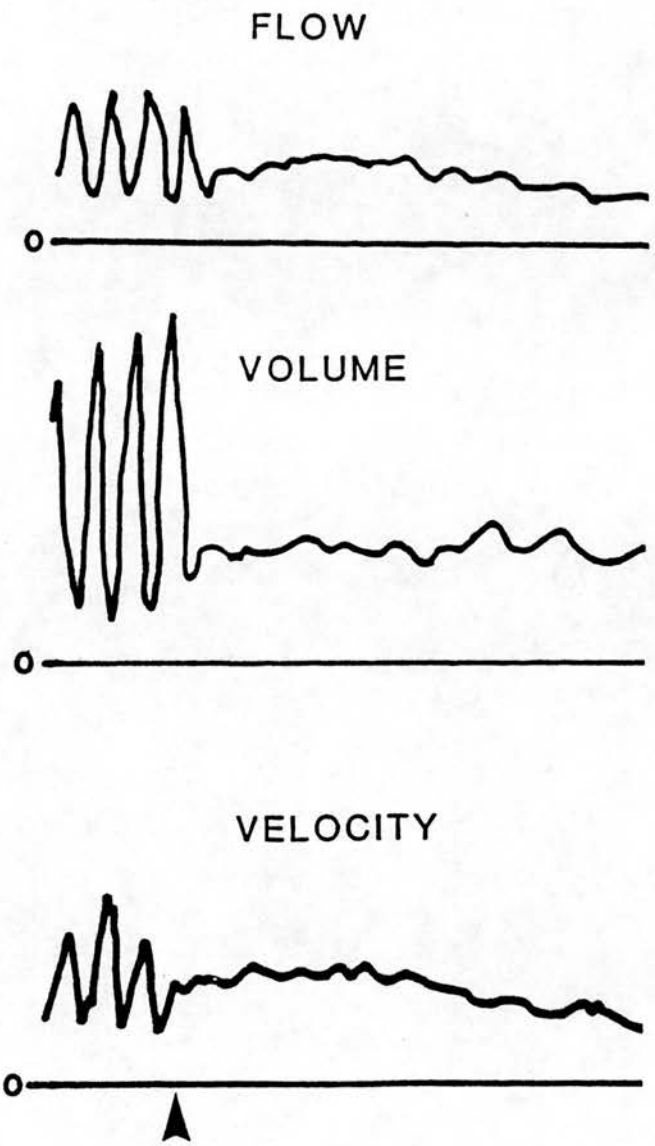


Figure 3.2. During ventilation, large fluctuations are seen in all LDF signals. These are prevented by stopping ventilation (arrow).

3.4 AMBIENT LIGHT CONDITIONS

Although ambient light levels in normal room lighting do not affect laser Doppler flowmeter signals, interference from the high intensity light of the bronchoscope was identified in these studies. Figure 3.3 shows an example of this problem. When the bronchoscope light was switched off while the LDF probe remained at a single site, there was an immediate increase in the volume, and thus the flow, signal. A 90% reduction in LDF signals during application of an external bright light has also been observed in studies of rat renal cortex (Lindsberg et al, 1989).

There are two possible explanations for this observation. The first, and most likely, explanation is that a portion of the high intensity light from the bronchoscope is detected by the LDF receiving fibre and photodetector. The detector operates at a wavelength of 780-800nm, just above the upper limit of the visible light spectrum (400-700nm), and within the spectrum of light generated by the bronchoscope. When this light enters the photodetector, it alters the ratio of the Doppler shifted to non-shifted photons, resulting in spuriously low volume, and thus flow, signals. When the bronchoscope light is switched off, the true volume and flow signals are seen.

An alternative explanation is that the presence of light from the bronchoscope causes a true alteration in blood flow in the microvascular bed. It has been shown that light, both in the ultraviolet and in the visible range, has an effect on vascular smooth muscle (Furchgott et al, 1961), this having been demonstrated in rabbit aorta and in canine carotid and femoral vessels. The phenomenon is most marked with light in the ultraviolet range, 310-420nm wavelength. The mechanism is unknown, though it has been suggested that light may induce the formation of a vasoactive

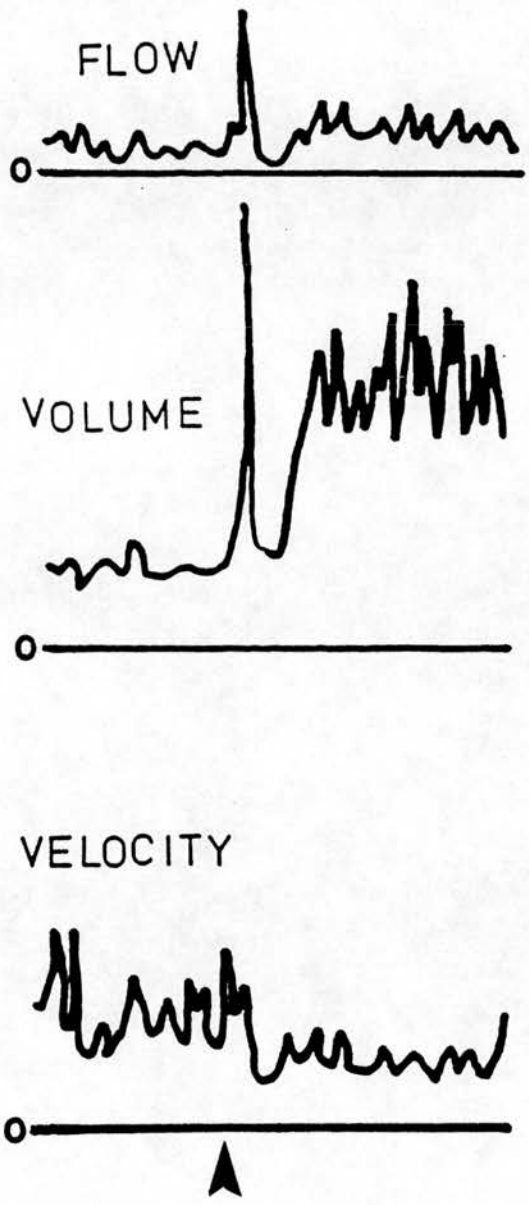


Figure 3.3. The effect of light from the bronchoscope on the LDF signals is shown. When the light is switched off (arrow), there is an immediate alteration in both the volume and velocity signals, with a resultant increase in the flow signal.

substance (Furchgott, 1971). Whether this phenomenon occurs in mucosal microvascular beds is unknown. However, in the present studies, the rapid time course of the response to switching off the high intensity bronchoscope light suggests that the former explanation is the more likely one for this observation.

It is theoretically unlikely that light transmitted by the laser Doppler flowmeter itself will induce alterations in blood flow, since it transmits light at a wavelength of 780nm, closer to the infrared than the ultraviolet range. This has been examined by Shepherd et al (1987), who studied the effect of the LDF system used in the present studies on blood flow in a hamster cremaster muscle prepared for intravital microscopy. They found that exposure to the laser light did not alter arteriolar diameter, nor did the presence or absence of laser light have any effect on the response of the arterioles to topically applied epinephrine.

Whatever the explanation for the change in laser Doppler signal with changing bronchoscopic light conditions, the problem was avoided in the present studies by reducing the bronchoscope light intensity to the minimum necessary for probe positioning in each study, and then checking that there was no alteration in signal as the light source was switched on and off. Thereafter, the bronchoscope light remained on, at this low intensity, to allow observation of the probe tip during recording.

3.5 CILIARY AND TISSUE MOTION

An observation of the present and of previous studies is that irregular fluctuations in the laser Doppler signal, which do not appear to correspond to tissue perfusion, occur even in the absence of the previously discussed artifacts. These irregular fluctuations in

the signal have been attributed to tissue motion. Kiel et al (1985), studying gastric mucosal blood flow using a helium-neon laser Doppler flowmeter, found that fluctuations in the laser Doppler flow signal were synchronous with electrical activity in the muscularis. However, even when the animal is dead, or when the vascular perfusion pressure is reduced to zero, a small signal persists. The source of this signal has not been fully explained.

In order to examine this further using our LDF system, we studied laser Doppler flow signals in the trachea of a pig before and after death. Following induction of anaesthesia using intravenous pentobarbital, catheters were inserted into the left ventricle and the pulmonary artery for measurement of vascular pressures. A tracheostomy was performed and the laser Doppler probe was inserted into the upper portion of the trachea, 3cm distal to the tracheostomy. Ventilation was stopped temporarily, and stable baseline recordings of blood flow, volume, and velocity were obtained. The pig was then killed by an intravenous overdose of sodium pentobarbital, while the laser Doppler signals were continuously monitored.

The results are shown in figure 3.4 (a). After the pentobarbital overdose, there was an initial sharp rise in both pulmonary artery pressure and in the laser Doppler signals, despite a progressive fall in left ventricular pressure. This suggests that the tracheal vascular resistance fell, perhaps due to the effects of the anaesthetic or due to hypoxia or hypercarbia.

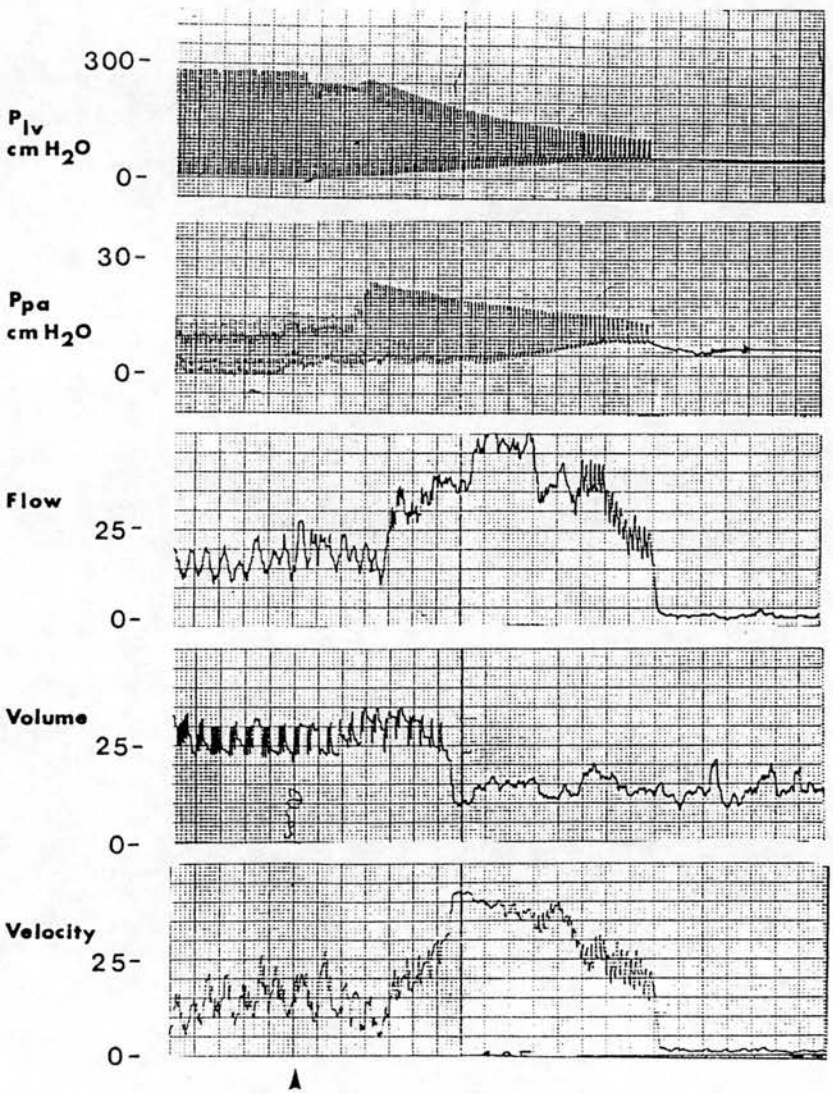


Figure 3.4 (a). Pentobarbital overdose was administered at point indicated by arrow. Thereafter, left ventricular pressure (Plv) fell, and pulmonary artery pressure (Ppa) rose and then fell. There was an initial rise in the LDF signal followed by a fall, but a small LDF signal persisted after cardiac contraction ceased.

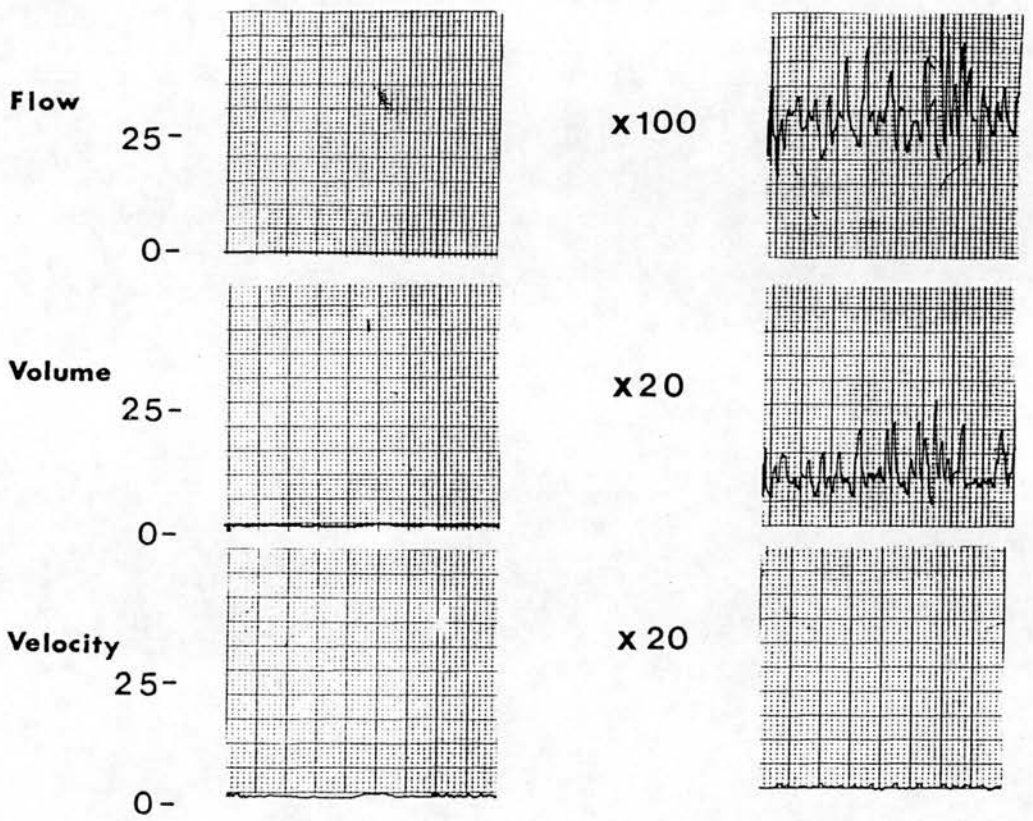


Figure 3.4 (b). Recordings obtained after excision of the trachea. A small flow signal persisted, mainly related to a volume signal, indicated in the left panel, and shown in the right panel at increased gain setting.

Thereafter, there was a fall in the laser Doppler signal, but even when cardiac contraction ceased, a small LDF signal persisted. The segment of trachea was then excised, opened by dissecting down the posterior membraneous portion, and pinned out, and the laser Doppler probe was repositioned over the same site which had been studied in the intact animal. A small 'flow' signal persisted (figure 3.4 (b)) and this was almost entirely attributable to a volume signal, with a very low velocity signal. The magnitude of this signal relative to the in vivo blood flow signal was small, less than 2%. When the preparation was re-examined after one hour, the LDF signal was zero.

There are several possible explanations for these observations. Tissue contractile activity, referred to above, may persist after death and excision of tissue. Ciliary beating, which normally occurs in the airway at a frequency of 12-15 Hz (Wanner, 1986), may also persist. This would be detected by the laser Doppler system, modifications of which have recently been used to study ciliary action in rabbit trachea (Verdugo & Golborne, 1988). In their studies, spectral analysis of the output from their laser Doppler spectroscope revealed a broad band low frequency (0 - 8Hz) component related to blood flow, and a higher frequency (13Hz) peak related to ciliary beating. The former band was abolished when the circulation was arrested, and the latter peak was abolished by treatment with Vanadate, which inhibits ciliary beating. The range of ciliary beat frequencies observed was 6 - 40 Hz, with higher frequencies being induced by treatment with isoprenaline. An alternative explanation for LDF signals occurring at zero flow would be Brownian motion of cells in the microvasculature. This has been suggested as the explanation for the occurrence of an LDF signal when a cell suspension is examined in a cuvette without flow (Shepherd et al, 1987).

Whatever the explanation, the small magnitude of the signal suggests that it is unlikely to introduce significant error into the blood flow measurements, except at very low flows. This will be further discussed in chapter 5.

3.6 ADDITIONAL SOURCES OF ARTIFACT

Some other potential sources of artifact must be considered. Theoretically, the level of oxygenation may affect the signal through its influence on the light absorbing properties of haemoglobin. In a study using a helium-neon laser, a minor effect on the signal of altering oxygenation was observed (Nilsson et al, 1980). With the infrared laser used in this study, however, variations of haemoglobin saturation from 0 to 100% have been shown to have no effect on the signal (Shepherd et al, 1987). This reflects the fact that the laser diode used in the present studies produces a reference beam in which the wavelength is distinct from the isobestic point for haemoglobin.

Another potential source of error is fibre movement artifact (Newsom et al, 1986). This cannot be eliminated with the present system, but can be minimised by holding the probe, particularly the portion leading from the bronchoscope to the signal processor, absolutely still during measurements. This problem will be discussed in chapter 8.

Having identified the above potential sources of artifact, measurement technique was modified accordingly to minimise errors and artifacts. The following chapters describe studies performed in dogs, sheep and huumans to examine the characteristics of LDF measurements in the airways, and to compare the laser Doppler system with other established methods of measuring airway wall blood flow.

CHAPTER 4

DETECTION OF ACUTE CHANGES IN AIRWAY WALL BLOOD FLOW IN DOGS BY LASER DOPPLER FLOWMETRY

4.1 SUMMARY

Measurements of tracheal wall blood flow were obtained by laser Doppler flowmetry in 7 dogs during fibreoptic bronchoscopy. Responses to acute changes in systemic blood pressure, produced by inflating a balloon in the inferior vena cava, were examined in six dogs. The mean slope of the flow-pressure relationship was 0.074 ± 0.078 LD units/cm H_2O and the mean pressure at zero flow, obtained by extrapolation of the flow-pressure plot was 34 ±41 cm H_2O. Tracheal wall blood flow responses to acute changes in airway pressure from 0-25 cm H_2O were examined in four dogs. Increasing airway pressure caused a reduction in tracheal wall blood flow. The LDF data suggest that this was due to both a reduction in perfusion pressure and an increase in vascular resistance. These data are comparable to those obtained in other studies using invasive methods of measurement, and suggest that laser Doppler flowmetry can be used to demonstrate acute changes in airway wall blood flow.

4.2 INTRODUCTION

Bronchial arterial blood flow is known to be influenced by changes in systemic arterial perfusion pressure and airway pressure, as discussed in chapter 1. Since laser Doppler flowmetry should provide continuous measurements of blood flow, the objective of this study was to examine laser Doppler signals measured in the central airways of dogs during manouevres designed to acutely alter blood flow, namely acute changes in perfusion pressure (induced by inflation of a balloon in the inferior vena cava) and in airway pressure (induced by inflation and deflation of the lungs with a positive and negative pressure device). In this study, LDF was not directly compared with other methods of measuring bronchial blood flow.

However, by deriving flow-pressure relationships using the laser Doppler signals, comparisons with previously published data were possible.

4.3 METHODS

Seven mixed breed dogs, mean weight 21 ± 3kg, were studied. The dogs were anaesthetised using intravenous pentobarbital sodium, 25mg/kg, paralysed using pancuronium bromide, 0.1 mg/kg, and studied in the supine position. Repeated small additional doses of pentobarbital sodium and pancuronium bromide were given as necessary to maintain anaesthesia and paralysis. The dogs were intubated and ventilated at a rate of 10-15 breaths/minute and a tidal volume of 15 ml/kg. End tidal carbon dioxide tension was continuously monitored using an infrared analyser (Hewlett Packard, Capnometer, model 47210A), and arterial blood gas samples were obtained during the procedure to ensure adequate ventilation and oxygenation. Airway pressure was measured using a differential pressure transducer (MP45 Validyne, Engineering Corp., Northridge, Ca.) attached to a side port of the endotracheal tube. Inspired air temperature (T_i) was measured using a copper constantin thermocouple (0.005 inch diameter wire - 95% response time in air ~ 0.5 sec.) mounted in the inspiratory limb of the ventilator circuit. Inspired air was 100% humidified by using an ultrasonic nebuliser (Devilbiss 65, setting 1-2), and was passed through a series of copper coils mounted in a heated water bath to warm it to approximate body temperature (37-40°C). A thermistor-tipped, triple lumen catheter was inserted into the main pulmonary artery under fluoroscopic control to allow measurements of pulmonary arterial pressure (P_{pa}) and determination of cardiac output by the thermodilution method. Catheters were also inserted into the right

femoral artery and vein for blood sampling and drug administration, and into the abdominal aorta, via the left femoral artery, for measurement of systemic arterial blood pressure (BP). All pressures were referenced to the level of the left atrium. P_{pa} and BP were continuously recorded on an 8-channel, strip-chart recorder (Hewlett Packard, type 7758A). A size 8F Fogarty balloon catheter (Edwards Laboratories, Santa Ana, California) was inserted into the inferior vena cava via the left femoral vein, to allow alteration of systemic arterial pressure by inflation and deflation of the balloon.

LDF measurements were obtained as described in chapter 2. A fibreoptic bronchoscope was introduced into the trachea through an airtight seal in the endotracheal catheter mount. The tip of the LDF probe was advanced 2-3 cm beyond the tip of the bronchoscope and was positioned, under direct vision, at sites over the lateral walls of the trachea in its mid portion, 8-12 cm from the main carina. The light intensity of the fibreoptic bronchoscope was reduced to a level at which there was no interference with the LDF signals, which were obtained during temporary cessation of ventilation. Flow, volume, and velocity signals were displayed on the 8-channel recorder.

4.4 PROTOCOL

4.4.1 Varying Perfusion Pressure

To examine the effects of varying systemic perfusion pressure on LDF signals, the ventilator was temporarily stopped and the balloon catheter in the inferior vena cava was inflated and deflated over a period of 10-20 seconds (figure 4.1). Airway pressure during this manoeuvre was zero cm H_2O. Blood flow, volume, and velocity in the tracheal wall were continuously recorded by LDF. A total of 20 flow-

pressure studies were obtained in 6 dogs. In the seventh dog, satisfactory placement of the balloon in the inferior vena cava could not be achieved.

4.4.2 Varying Airway Pressure

To examine the effects of varying airway pressure on LDF signals, a device was used which incorporated a positive and negative pressure gas supply, allowing the lungs to be slowly inflated and deflated over a period of 10–40 seconds. The ventilator was temporarily disconnected, and the inflation/deflation device connected to the endotracheal catheter. The lungs were inflated to an airway pressure of 25 cm H_2O and then deflated slowly (figure 4.2). The mean $\pm$ SD duration of inflation was 8 $\pm$ 2 seconds, and of deflation was 16 $\pm$ 6 seconds. Blood flow, volume and velocity were continuously recorded by LDF, and vascular pressures were monitored. A total of 19 such manoeuvres were performed in 4 dogs. Two dogs were studied before the inflation/deflation device was available, and the other dog died before this part of the protocol could be performed.

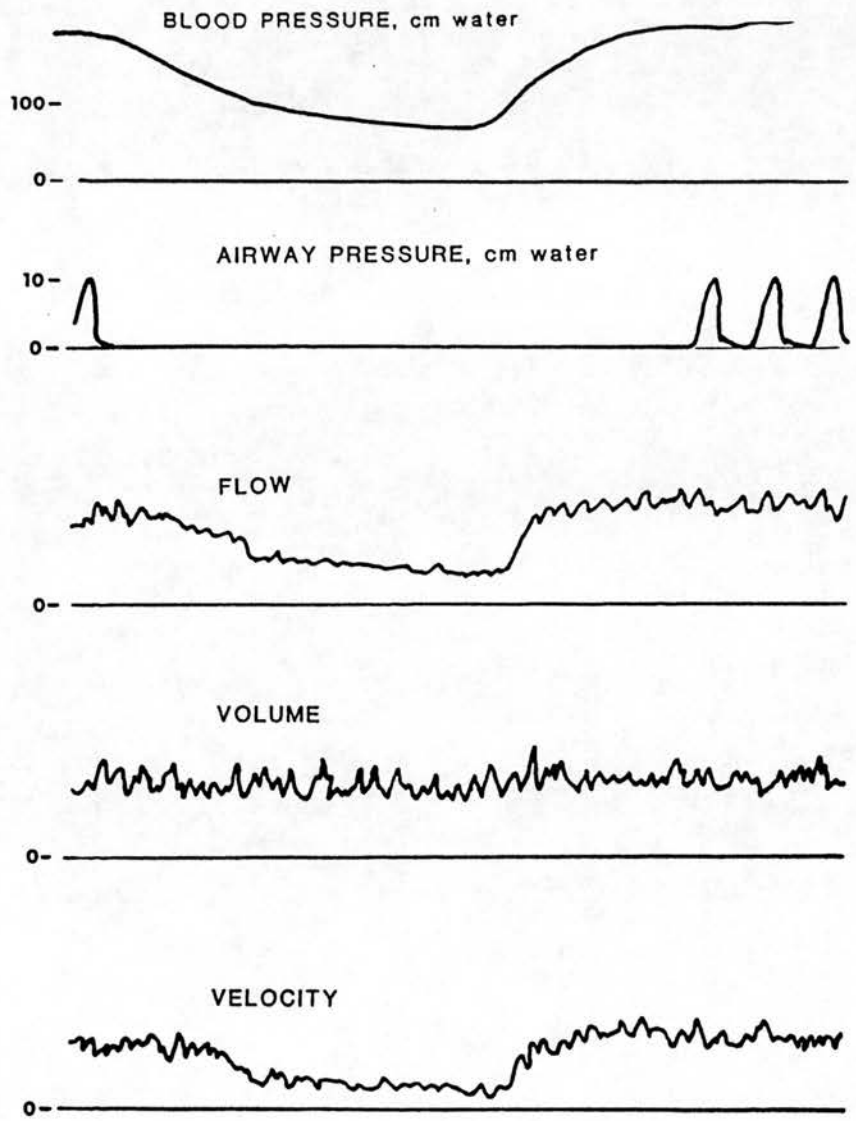


Figure 4.1. An example of the changes in mean systemic blood pressure (cm H_2O) and laser Doppler signals during inflation and deflation of a balloon in the inferior vena cava. Measurements are made during cessation of ventilation (airway pressure = 0 cm H_2O).

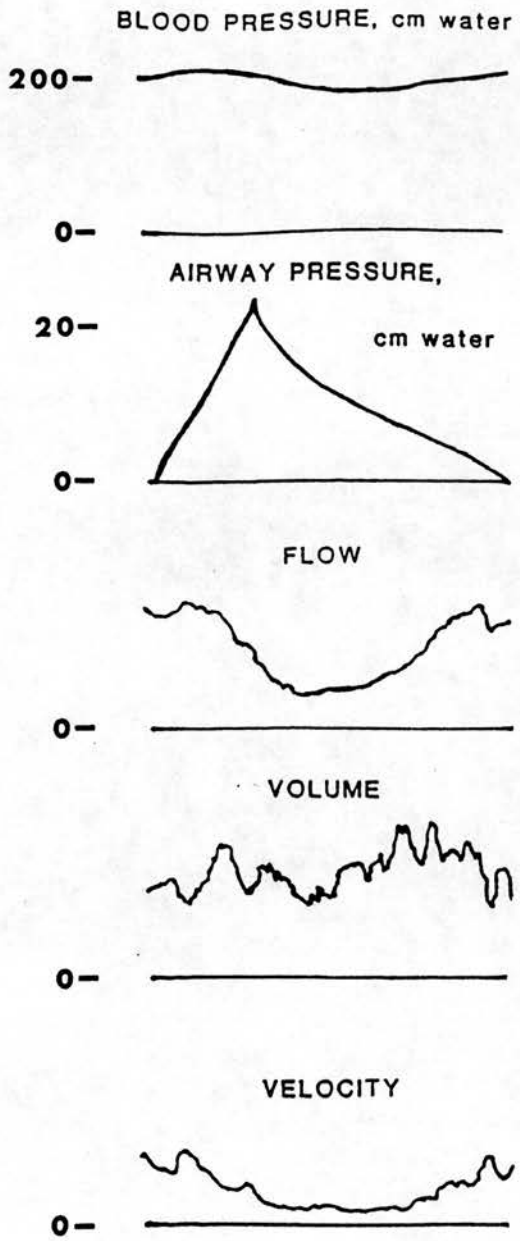


Figure 4.2. An example of the changes in mean systemic blood pressure and laser Doppler signals during an acute change in airway pressure from 0 - 25 cm H_2O.

4.5 DATA ANALYSIS

In order to plot relationships between pressure and flow, volume, and velocity, the data from the strip-chart recordings were transferred into a form useable by the Lotus 1-2-3 system (Lotus Development Corp., Cambridge., Ma.), by the use of a manual digitizing tablet (GTCO, Rockville, Md., USA). The serial data from the digitizer were fed to a personal computer of the IBM variety. An algorithm was used to correct for the delay produced by the electronic circuit to derive mean blood pressure, and a further correction was used to allow for the phase lag inherent in the laser Doppler apparatus.

For the first series of experiments, tracheal blood flow, volume, and velocity were plotted against mean systemic blood pressure. Separate plots were made for increasing and decreasing blood pressure. Linear regression analysis was applied to the flow-pressure relationships allowing derivation of incremental vascular resistance (slope), and, by extrapolation to the X-axis, of the pressure at zero flow.

Analysis of the second series of experiments was more difficult since continuously changing airway pressure resulted in continuous changes in inflow and outflow pressure and vascular resistance throughout the manoeuvre. In this situation, incremental vascular resistance cannot be determined (Mitzner & Chang). However, if the relationship between inflow pressure and blood flow is examined, a value for instantaneous vascular "resistance" can be derived for each individual point on the flow-pressure curve. This is not a true resistance, but simply a device employed to allow comparison of the flow/pressure curves obtained in each experiment over similar ranges of pressure.

We wished to detect whether changes in airway wall blood flow during changes in airway pressure were simply due to changes in perfusion pressure, as in the first series of experiments, or whether some additional vasomotion or change in vascular resistance was also occurring during the airway pressure change. In order, therefore, to allow comparison of the second series of experiments with the first, tracheal blood flow was again plotted against mean systemic blood pressure, and, from this plot, values for instantaneous vascular "resistance" over the range of systemic blood pressure measured were derived. By constructing similar plots for the first series of experiments, in which perfusion pressure was changed by means of the inferior vena caval balloon, we were able to compare the magnitude of "resistance" changes which occurred during the two manoeuvres.

4.6 RESULTS

Blood gas tensions, temperatures and cardiac output prior to performing the manoeuvres are shown in table 4.1.

TABLE 4.1

DOG	1	2	3	4	5	6	7
P_{o2} (mmHg)	103	91	83	98	80	91	96
P_{co2} (mmHg)	33	35	39	38	35	40	32
pH	7.41	7.37	7.36	7.31	7.36	7.35	7.36
T_{insp} (°C)	30	26	32	31	37	37	33
T_{body} (°C)	38	39	39	37	39	38	37
CO (L/min)	2.89	2.77	2.63	2.66	2.37	3.46	2.99

4.6.1 Varying Perfusion Pressure

An example of the response of the blood pressure and LDF signals to inflation of the inferior vena caval balloon is shown in figure 4.1. The flow-pressure, volume-pressure and velocity-pressure plots generated from this data are shown in figure 4.3. Linear regression analysis of tracheal blood flow versus perfusion pressure revealed significant relationships for both decreasing and increasing pressure, r values ranging from 0.63 to 0.9, $p<0.001$. The mean flow-pressure plots, obtained during decreasing blood pressure, for each of the six dogs are shown in figure 4.4. The solid lines indicate the actual ranges over which flow and pressure were measured, and the dotted lines represent extrapolations from the data. When all curves from all the dogs were examined, the mean ($\pm$ SD) slope of the flow pressure relationship was 0.074 $\pm$ 0.078 LDF units/cm H_2O for decreasing pressure, and 0.074 $\pm$ 0.071 LDF units/cm H_2O for increasing pressure. The mean ($\pm$ SD) x-axis intercept (pressure at zero flow) was 34 $\pm$ 41 cm H_2O for decreasing blood pressure, and 34 $\pm$ 56 cm H_2O for increasing blood pressure. Examination of the volume-pressure and velocity-pressure plots showed that changes in flow were almost entirely attributable to changes in velocity except at low perfusion pressures (<90 cm H_2O), when changes in volume also occurred (figure 4.5).

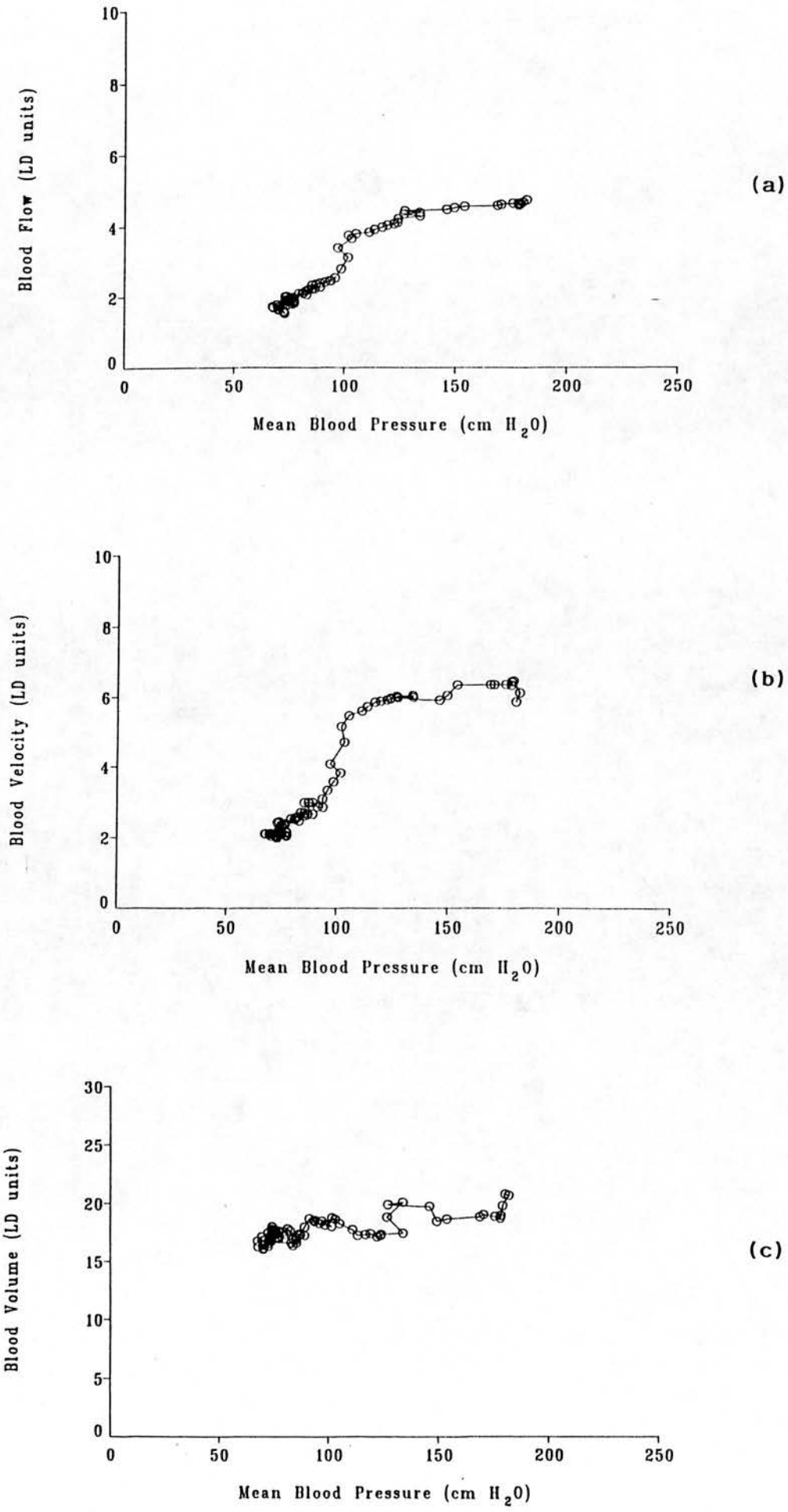


Figure 4.3. Plots from one representative dog showing the relationships between mean systemic blood pressure and a) blood flow, b) blood velocity, c) blood volume, measured by laser Doppler flowmetry. All measurements were made at constant airway pressure.

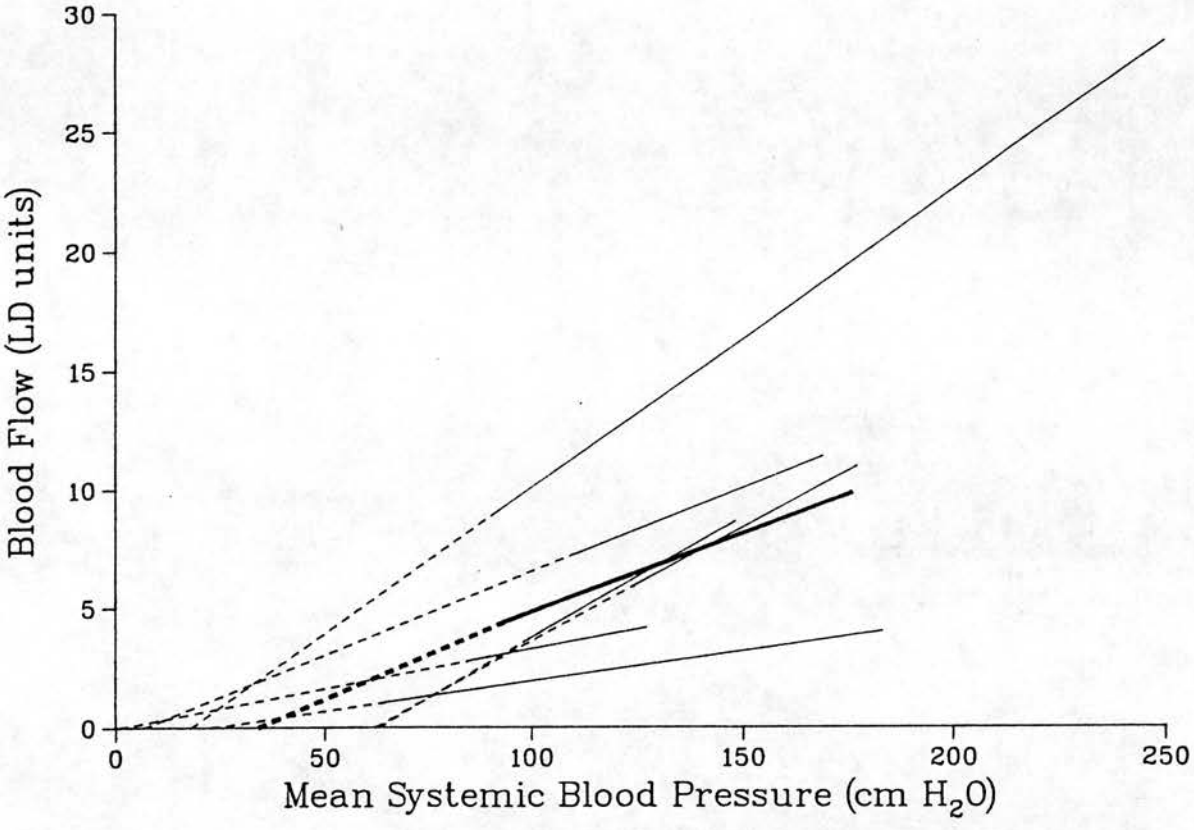


Figure 4.4. Average data from each of six dogs showing the relationship between tracheal blood flow and mean systemic blood pressure. The solid lines show the actual ranges over which flow and pressure were measured, and the dotted lines represent extrapolations to determine the pressure at zero flow. The thick line shows the overall mean data for the six dogs.

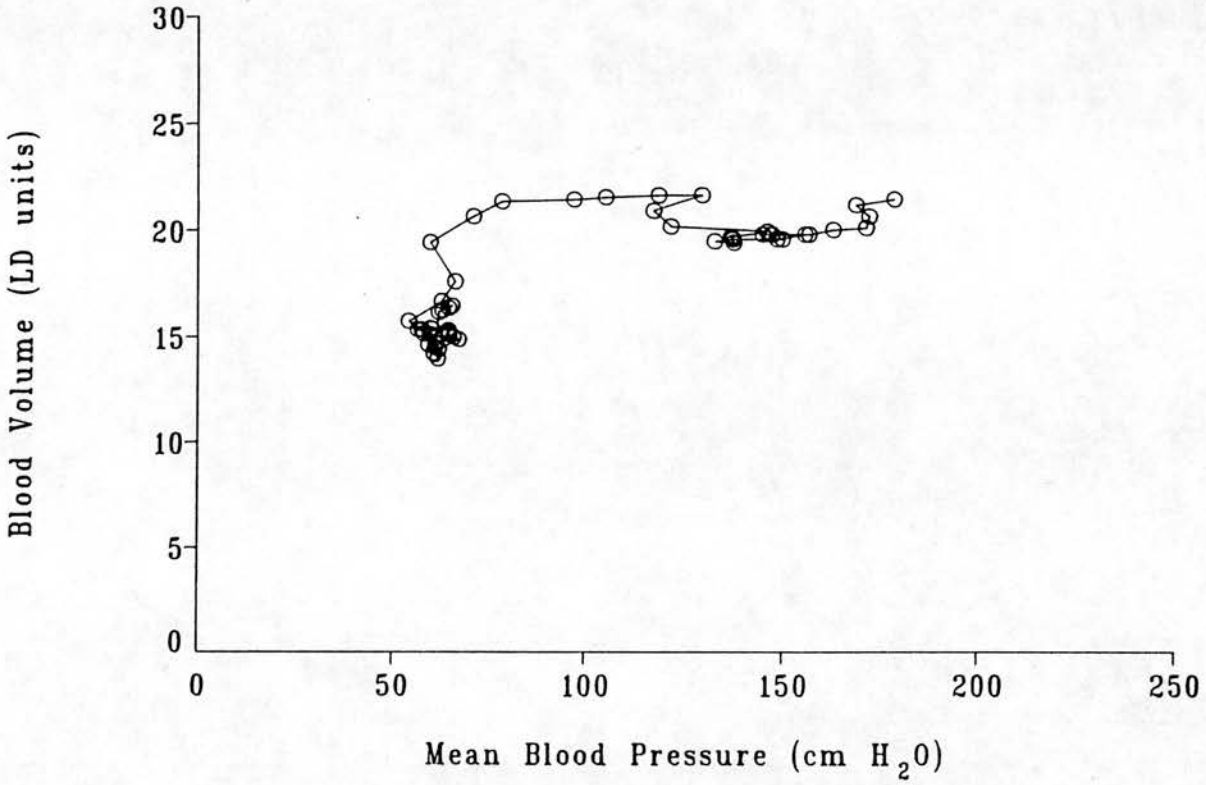


Figure 4.5. A single plot from one dog showing a fall in the tracheal blood volume signal at low perfusion pressures of less than 90 cm H_2O.

4.6.2. Varying Airway Pressure

Acute increases in airway pressure produced a pronounced fall in tracheal wall blood flow (figure 4.2), associated with a small fall in mean systemic blood pressure. The relationship between increasing and decreasing airway pressure and blood flow from one representative dog is shown in figure 4.6. The apparent hysteresis in this relationship is due to the presence of a physiological lag phase between changes in airway pressure and changes in mean systemic blood pressure.

The relationship between the change in instantaneous vascular "resistance" and mean systemic blood pressure during increasing airway pressure is shown in figure 4.7. Mean data are presented for each dog, and, for comparison, similar plots derived for each dog from the flow-pressure curves in figure 4.4 (i.e. during inflation of the balloon in the inferior vena cava) are also shown. The magnitude of the change in vascular resistance over the measured range of systemic blood pressure is much greater during the airway pressure change than during inflation of the inferior vena caval balloon. This suggests that active vasomotion is occurring.

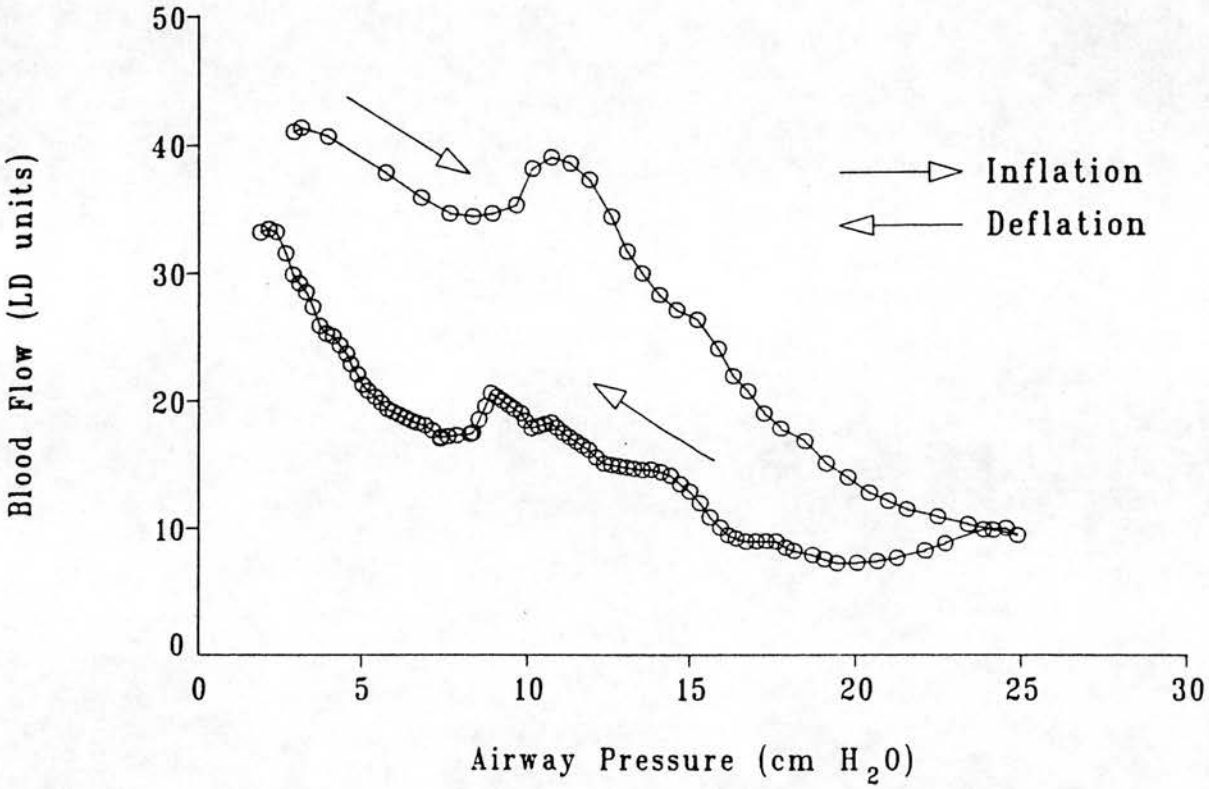


Figure 4.6. A single plot from one dog showing the effects of airway pressure changes on blood flow signals. The apparent hysteresis is related to the occurrence of a lag phase between changes in airway pressure and changes in mean systemic blood pressure.

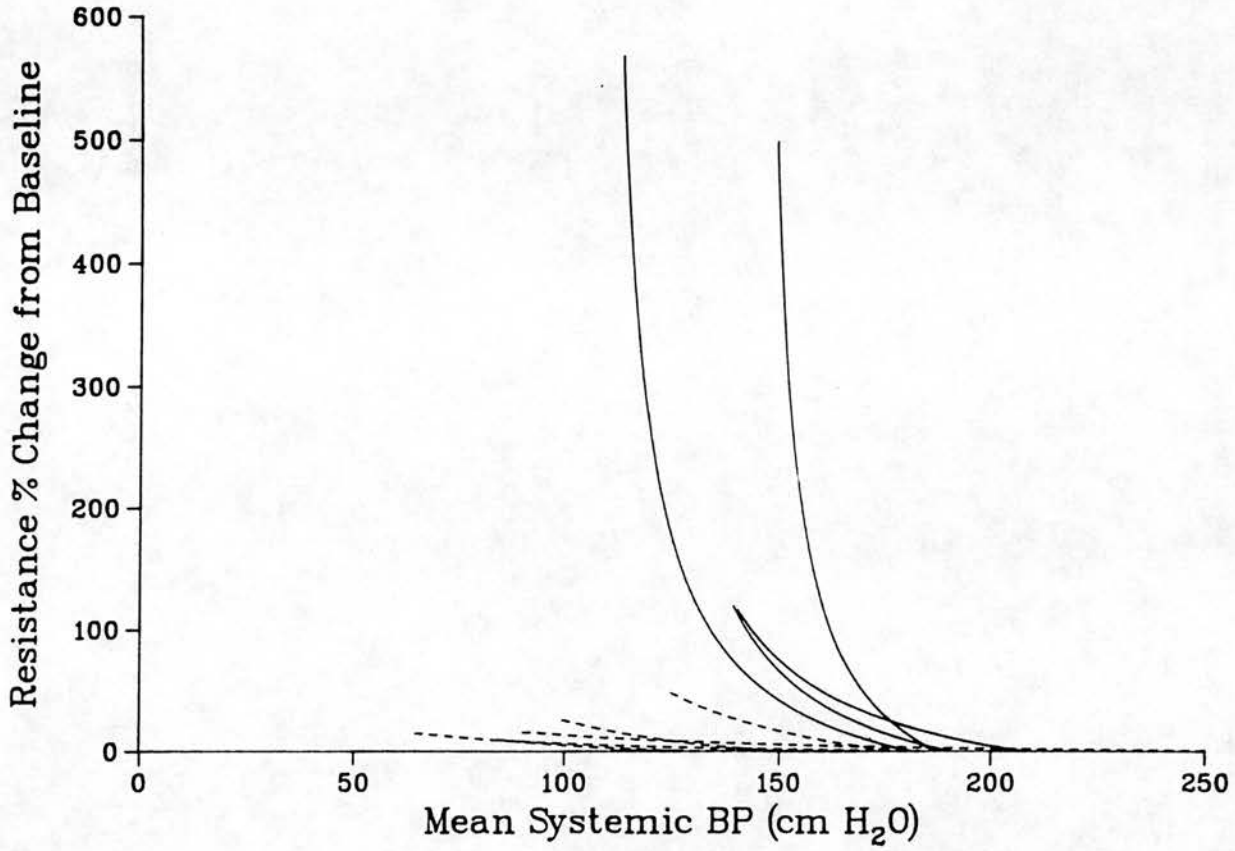


Figure 4.7. The relationship between instantaneous vascular "resistance", expressed as per cent increase from baseline, and mean systemic blood pressure during inflation of a balloon in the inferior vena cava (dotted lines) and during increasing airway pressure (solid lines). Baseline resistance was defined as the resistance calculated at the beginning of the manoeuvre, i.e. at maximum blood flow.

4.7 DISCUSSION

The flow-pressure data obtained in this study are similar to those previously observed in dogs and sheep using invasive techniques for measuring bronchial arterial blood flow (Baile et al, 1984, Baier et al, 1985, Cassidy & Haynes, 1986, Wagner et al, 1987). In those dogs in which perfusion pressure was altered by inflation and deflation of the inferior vena caval balloon, we found, by extrapolation, a mean pressure at zero flow of 34 cm H_2O, a value similar to that observed in sheep in the study of Wagner et al (1987). There was, however, considerable variation in the pressure at zero flow. Examination of figure 4.4 shows that 4 dogs had mean pressures at zero flow in the range of 1-23 cm H_2O, whilst 2 dogs had higher values of 62 and 63 cm H_2O. This is again similar to the data of Wagner et al (1987), who suggested that the range of zero-flow pressures found for the bronchial circulation may reflect variations in downstream pressure due to the dual venous drainage of the bronchial circulation. In addition, some of the variation may be due to non-linearity of the flow-pressure curve due to the compliant nature of the vascular bed in which the LDF probe detects blood flow (Mitzner, 1983). When the velocity-pressure and volume-pressure plots were examined, it was apparent that over the higher ranges of pressure, changes in flow were related principally to changes in velocity of cells. Only when pressures less than 90 cm H_2O were obtained did the volume signal begin to decline, suggesting that derecruitment of vessels was occurring. This observation raises the question of whether autoregulation may occur in the tracheobronchial vascular bed, and will be dicussed again in chapter 5 in relation to studies in sheep.

The effects of changing airway pressure on blood flow observed in

this study are also similar to previous observations. In a previous study, Baile et al (1984) found a significant reduction in blood flow to the bronchi and lung parenchyma during the sustained application of 15 cm H_2O of positive end-expiratory pressure (PEEP). There was also a trend towards reduced tracheal blood flow in that study, although it did not reach statistical significance. Their data showed that changes in blood flow were due to alteration in both perfusion pressure and vascular resistance. They compared the slopes of their pressure-flow curves, obtained during steady-state application of PEEP, with those of Salisbury et al (1957), who altered perfusion pressure only, in a heart-lung bypasss preparation in dogs. PEEP increased incremental vascular resistance over the same range of perfusion pressure by 270%. In another study, Cassidy and Haynes (1986) demonstrated a three-fold increase in bronchial vascular resistance during the application of 10 cm H_2O PEEP in a lung in which the pulmonary artery had been occluded. In the study of Wagner et al (1987), increasing mean airway pressure in sheep from 4.8 to 14.1 mm Hg also increased bronchial vascular resistance, although by a smaller magnitude of about 50%.

The data from the present study cannot be directly compared with those from these previous studies, since, in the present protocol, inflow and outflow pressure and vascular resistance were changing simultaneously. However, in the present studies, during increasing airway pressure, the observed fall in tracheal blood flow was greater than that which would be expected simply from the fall in mean systemic (inflow) blood pressure (Figure 4.6). This was probably due to increases in both vascular resistance and outflow pressure.

Theoretically, an increase in outflow pressure alone could account for the entire fall in blood flow observed without requiring a

change in vascular resistance (Mitzner, 1983). To assess whether this might be the case, right atrial pressure, the appropriate downstream pressure for the tracheal vessels, was measured during both manoeuvres in one dog. The magnitude of right atrial pressure changes throughout both manoeuvres was small. During inflation of the balloon in the inferior vena cava, right atrial pressure decreased by a maximum of 4 cm H_2O. This would partially offset the fall in blood flow through the vascular bed related to the fall in inflow (systemic) pressure. During increases in airway pressure to 25 cm H_2O, the right atrial pressure rose by a maximum of 6 cm H_2O, and this would contribute to the fall in blood flow observed. However, these small changes in downstream pressure are unlikely to entirely account for the change in blood flow observed during the manoeuvres. To test this, the data from this dog was re-analysed in a fashion similar to that shown in figure 4.6, but using perfusion pressure rather than mean systemic blood pressure, where perfusion pressure was defined as mean systemic blood pressure minus right atrial pressure for the first study, and as mean systemic blood pressure minus right atrial pressure minus airway pressure for the second study. Subtracting both airway pressure and right atrial pressure allows for either or both to be the relevant downstream pressure. Over similar ranges of perfusion pressure (from 170 cmH_2O to 80 cmH_2O), the rise in vascular "resistance" was 140% from baseline in the first study (no airway pressure change), and 450% from baseline in the second study (airway pressure rising from 0 to 25 cmH_2O). These results suggest that increases in airway pressure reduce blood flow to the tracheal wall not only by decreasing perfusion pressure and but also by increasing vascular resistance.

The mechanism by which increasing airway pressure increases vascular resistance is unknown. It could be related to lung

inflation, leading to mediator release, or to mechanical factors. Baier et al (1985) have shown that cyclooxygenase products are released during lung inflation, but are not responsible for the changes in bronchial blood flow. Mechanical factors may therefore be important. The tracheal and bronchial vessels course along the outer surface of the bronchial tree and radicals derived from the major vessels penetrate the muscular layer of the airway wall to supply the airway mucosa. It has been suggested that during lung inflation, the longitudinal vessels could be stretched and the penetrating vessels distorted due to distension of the airway wall (Wagner et al, 1987). The results of this study may shed some further light on this subject. The laser Doppler probe used in this study is unique in that it provides separate information on the volume and velocity components of blood flow. Theoretically, one would expect that a change in the resistance upstream to the vascular bed under study would result in altered velocity of red cells, whilst recruitment or derecruitment of the vascular bed would primarily alter the volume of moving red blood cells. In this study, the changes in blood flow were almost entirely due to changes in velocity. It is thought that LDF measures blood flow in a volume of about 1 mm^3 (Bonner & Nossal, 1981), which, in the airway, would mean that the blood flow measurement is derived predominantly from the submucosal plexus of vessels (Laitinen et al, 1986a). If this is true, the results of this study would suggest that the change in resistance occurred upstream to the submucosal plexus, and this would be compatible with the observations of Wagner et al (1987). They used a resistive model of the bronchial vasculature to explain the effects of PEEP which they had observed in sheep using the isolated, cannulated branch of the bronchial artery. They concluded, on the basis of examining pressures at zero flow, that the likely site

of altered vascular resistance during PEEP was in the penetrating vessels connecting the submucosal with the peribronchial plexus.

In this study, it has been shown that acute alterations in airway wall blood flow can be detected by laser Doppler flowmetry when the LDF probe is applied at one site during bronchoscopy. Pressure-flow studies for the airway wall vasculature obtained using this technique are comparable to those obtained using more invasive techniques. The nature of the data obtained may provide some further insight into the mechanism of the pressure-flow responses. However, a simultaneous comparison of laser Doppler flowmetry with more invasive techniques has not been performed. The following chapters describe studies designed to directly compare the technique of laser Doppler flowmetry with other methods of measuring airway wall blood flow.

CHAPTER 5

MEASUREMENT, BY LASER DOPPLER FLOWMETRY, OF CHANGES IN AIRWAY WALL BLOOD FLOW INDUCED BY CHANGING BRONCHIAL BLOOD FLOW IN SHEEP

5.1 SUMMARY

In this study, laser Doppler flowmetry was used to measure tracheal and bronchial wall blood flow in anaesthetised, open-chest sheep during bronchoscopy. The response of the LDF flow signals at 4 regions of the airway walls to varying bronchial arterial flow rates was examined in both live and dead sheep by cannulation and subsequent perfusion of the common bronchial artery at varying flow rates using a roller pump. Ten sites were examined in each region. In the live sheep, variations in bronchial arterial blood flow resulted in variations in LDF signals in the principal bronchus, and in lobar and segmental bronchi, but not in the trachea. In the dead sheep, variations in bronchial arterial blood flow resulted in variations in LDF signals in all four regions. Within regions, the average response of the LDF signals to varying flow was approximately linear in both live and dead sheep, but considerable site to site variation in response was observed. In the live sheep, a substantial LDF signal was present even when the bronchial artery flow was set to zero, and when the bronchial artery was perfused with dextran solution, which would, in theory, be expected to produce no LDF signal. A very small signal was also detected in the dead sheep under zero flow conditions. These observations suggest that laser Doppler flowmetry, in addition to detecting blood flow from the bronchial artery, also detects background noise and/or collateral circulation.

5.2 INTRODUCTION

In the previous chapter, it was demonstrated that acute changes in blood flow to the airway walls of dogs could be demonstrated during bronchoscopy using laser Doppler flowmetry. The purpose of this study was to measure relative changes in blood flow to the central airways of sheep, using a preparation which allowed a direct comparison of LDF signals with bronchial arterial blood flow. The sheep was chosen for this study because of the anatomy of its tracheobronchial vasculature.

About 70% of the systemic blood supply to the lungs of sheep is derived from the ramus bronchalis communis (common bronchial artery), a branch of the A.bronchoesophagea (bronchoesophageal artery (Magno et al, 1982, 1987, Charan et al, 1987). Cannulation of this vessel in open-chest sheep allows the blood flow in the bronchial vascular bed supplied by this vessel to be varied. By monitoring the bronchial arterial pressure during variations in flow, vascular resistance can also be derived (Wagner et al, 1987). This model has been used previously to examine the effects of positive-end-expiratory pressure (Wagner et al, 1987), and hypoxia (Wagner et al, 1988), on vascular resistance in the bronchial circulation. In this study, using this preparation, blood flow into the common bronchial artery of sheep was varied while simultaneous LDF measurements of blood flow in the trachea and major bronchi were obtained. The effects on the LDF measurements of varying the blood flow into the common bronchial artery were examined in live sheep. The protocol was also repeated immediately after the death of the sheep, in order to examine the response in the absence of potential sources of collateral blood flow.

5.3 METHODS

Four male sheep of mixed breeds, weighing 23-30 kg, were studied in the right lateral position. After induction of anaesthesia using ketamine (1g IM), the sheep were tracheostomized and ventilated at a rate of 15 breaths per minute, and tidal volume of 10-12 ml/kg. Supplemental oxygen was given to maintain P_aO_2 greater than 90 mmHg, and airway pressure was measured from a side port in the tracheal catheter. Five cm H_2O of positive end-expiratory pressure was applied to maintain lung inflation during open-chest conditions. Anaesthesia was maintained using a continuous infusion of sodium pentobarbital (15mg/kg loading dose, 20 mg/kg hourly dose) and paralysis using intravenous pancuronium bromide (2mg IV) as required. Atropine (1 mg IM) was given to reduce airway secretions. Catheters were inserted into the right and left femoral arteries for measurement of blood pressure and withdrawal of blood to supply the bronchial artery perfusion pump. Another catheter was inserted into the femoral vein for blood sampling and drug administration.

A left lateral thoracotomy was then performed between the fifth and sixth ribs. The left hemiazygous vein was tied off and divided upstream from the point of entry of the bronchial vein to allow access to the common bronchial artery. This artery was isolated and the ramus oesophagus (oesophageal branch) and the ramus trachealis thoracicus (thoracic tracheal branch) were tied off. After giving an intravenous dose of heparin, 40,000 units, the bronchial artery was cannulated using a 16 guage angiocatheter, which was then flushed with heparinized saline (0.9% solution), and connected to a variable speed roller pump (Gilson, model F117-374), which was used to pump blood from the femoral artery catheter into the bronchial artery. Bronchial arterial pressure was measured at a side arm of the cannula. A

control rate of bronchial bood flow of 0.6 ml/kg body weight/minute was chosen, since this has been shown to be the average normal blood flow in this vessel (Charan et al, 1985), and flow was varied to twice and to half of the control value, and to zero (see later).

LDF measurements of blood flow were made as previously described in chapter 2. The LDF probe was introduced into the airways through the suction channel of a flexible bronchoscope (Olympus IT10), which was inserted through an airtight seal in a Y connector attached to the tracheostomy tube. To obtain blood flow measurements, the tip of the probe was advanced, under direct vision, to a position 2-3 cm beyond the tip of the bronchoscope and allowed to rest on the mucosal surface. The light intensity of the fibreoptic bronchoscope was reduced to prevent interference with the LDF signal, and measurements were made only during cessation of ventilation to avoid artifacts due to respiratory movements (see Chapter 3). An averaging time of 1 second was used, and flow, volume and velocity signals were displayed on a twelve-channel hot pen recorder.

5.4 PROTOCOL

The first objective of this study was to examine the effects of changing the rate of blood flow perfusing the bronchial artery on the blood flow signals measured by LDF, at various regions in the central airways. Four regions were chosen for LDF measurements (figure 5.1). These were the posterior wall of the trachea between the origin of the tracheal bronchus and the main carina (region 1), the medial wall of the left principal bronchus (region 2), the medial wall of the left lower lobe bronchus (region 3), and the proximal portions of two lower lobe segmental bronchi (region 4). After positioning the LDF probe,

the blood flow to the bronchial artery was set at twice the control value, and an LDF measurement was made during brief cessation of ventilation (~5 sec). During this period, the measurement remained stable (figure 5.2). The bronchial blood flow was then reduced in a stepwise fashion to the control value, half control, and zero, and an LDF measurement was made to correspond with each level of bronchial blood flow (fig. 5.2). The probe was then repositioned to another site within the same region and the procedure was repeated. A total of ten sites chosen at random were studied within each region. When measurements in one region had been completed, the LDF probe was advanced to the next, more distal, region, and the entire procedure was repeated. Before moving to the next region, the probe was removed from the bronchoscope and the tip cleaned of any mucus, the airways were suctioned, if necessary, to remove excess secretions, the arterial blood gases were measured, and blood pressure, airway pressure and bronchial arterial pressure were allowed to stabilise.

In two sheep (1 and 2), the effects on the LDF signal of perfusing the bronchial artery with a cell free solution of dextran were also examined at two sites in region 3 after completion of the first part of the protocol. In three sheep, (2, 3, and 4), the protocol of LDF measurements made during life while varying the bronchial blood flow was repeated after the sheep had been killed using an overdose of intravenous pentobarbital sodium. In the dead sheep, only two sites were studied in each of the four regions, since we were concerned that post mortem changes in the vessel walls and blood clotting may occur.

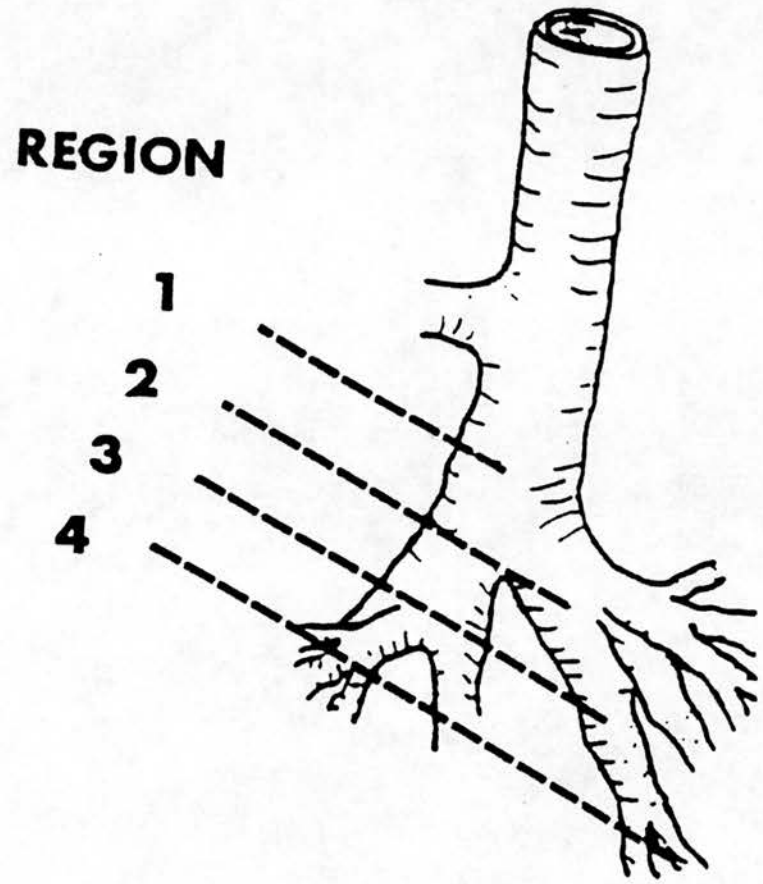


Figure 5.1. Diagram of the sheep trachea and bronchi (posterior view) indicating the regions in which the LDF measurements were made.

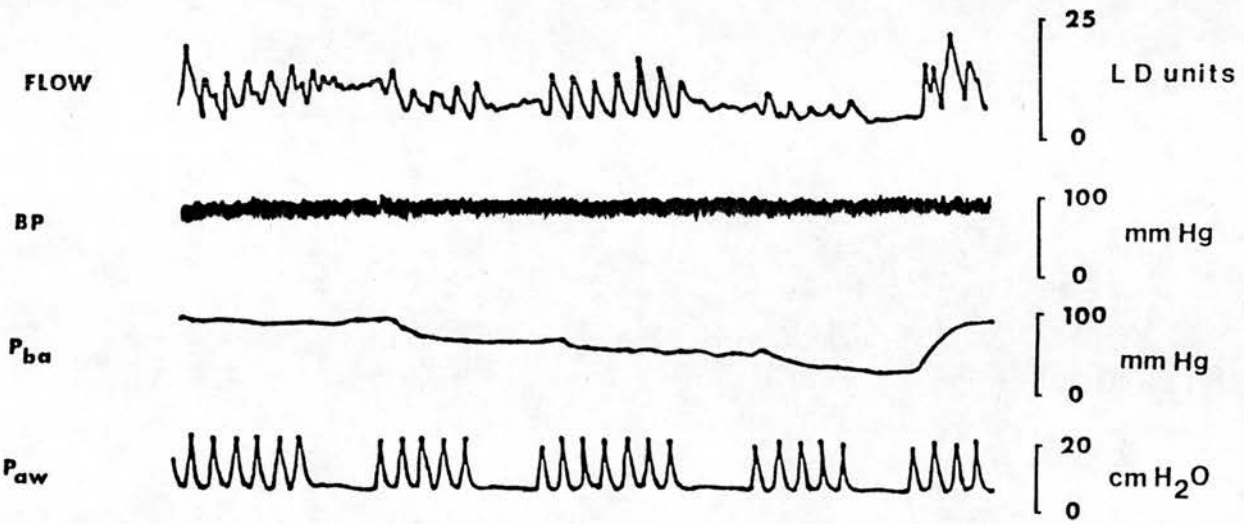


Figure 5.2. Actual tracing of the LDF response to changing bronchial arterial blood flow at one site in the left main bronchus of one sheep, during four levels of bronchial flow. Four periods of cessation of ventilation are indicated by the flat sections of the airway pressure trace (Paw), and changes in bronchial blood flow are indicated by changes in bronchial arterial pressure (P_{ba}).

5.5 DATA ANALYSIS

There was considerable variability between the LDF signals at different sites in all regions. Since there was a relationship between the average value and the standard deviation of the LDF flow signals for different regions, the square root of the LDF signals was used for statistical analysis. The transformed data were analysed using a single factor, repeated measures design (Winer, 1971), in which "bronchial blood flow" was the repeated measure, and "region" was the grouping factor. Within each region, there were ten random sampling sites and the factors of region and flow were treated as fixed effects. Each sheep was treated as a single repeated measures experiment. Within this design, it is possible to detect differences between sites attributable to a given region, differences within sites due to variation in flow, and region/flow interactions. A conservative test for significance, as proposed by Greenhouse and Geisser, was used (Winer, 1971). For all sheep, the effect of region between sites, the flow effects within sites, and the region/flow interaction were highly significant ($p<0.005$ to 0.001). Therefore, in order to examine the effects of changing region and flow, comparisons between regions must be made at constant flow, and comparisons between flows must be made within a constant region. These contrasts were regarded as significant if $p<0.05$. The analyses were done using BMDP/PC statistical software (BMDP Statistical Software, 1987).

5.6 RESULTS

There was considerable variation between sites, between regions and between sheep in the LDF measurements at control bronchial blood flow. The coefficient of variation (CV) between sites within regions was 56 $\pm$ 21 %, between regions within sheep the CV was 47 $\pm$ 21 %, and

between sheep the CV was 31%.

The effects of changing common bronchial arterial blood flow on the LDF flow signals in the live sheep are shown in figure 5.3. In region 1, there was no detectable effect of changing bronchial arterial blood flow on the LDF signal. In region 2, reducing bronchial arterial blood flow resulted in reduction ($p<0.05$) in LDF signal in all sheep. In regions 3 and 4, reducing bronchial arterial blood flow also resulted in reduction in the LDF signals, although, in some cases, the contrasts between adjacent flows did not quite achieve statistical significance. Of interest also was the nature of the LDF response as the bronchial arterial blood flow was altered in stepwise fashion from 200 per cent of control to zero. In the repeated measures analysis, the four repeated measurements can be partitioned into linear, quadratic, and cubic components. For both the single flow effect, and the flow-region interaction, the linear components were highly significant ($p<0.005$). The implication of this is that changes in bronchial arterial blood flow result in approximately linear changes in the LDF signal. It is however apparent that at the highest bronchial blood flow (i.e. at 200 per cent of control), for all the sheep, the LDF signals tended to be lower than would be expected from a truly linear relationship (figure 5.3). This will be discussed later.

An LDF signal was obtained in all regions even when the bronchial arterial pump flow was set to zero. This comprised, on average, 64 $\pm$ 14% (mean $\pm$ SD) of the control flow signal in region 2, 69 $\pm$ 11% in region 3, and 56 $\pm$ 20% in region 4. If this offset from zero flow is subtracted from all the LDF signals, and the LDF signals, thus derived, are expressed as percentages of the values at control flow, then the magnitude of change of LDF signals and bronchial arterial

blood flow is closer to, though not equal to, a one-for-one relationship, as shown in figure 5.4. Potential explanations for the offset at zero flow and for the pattern of LDF responses will be discussed later.

The effects on the LDF signals of changing common bronchial blood flow in the dead sheep are summarized in figure 5.5. There are two major differences when these data are compared with the data from the live sheep (fig. 5.3). First, changes in bronchial arterial blood flow now result in changes in LDF signals in all of the four regions. However, the absolute magnitude of the LDF signals at all levels of bronchial arterial flow are lower than in the live sheep, except in region 3, where, at the higher levels of bronchial arterial flow, the LDF signals in the live and dead sheep are of similar magnitude. Second, the LDF signal at zero bronchial arterial pump flow is now close to zero. Once again, the relationship between bronchial arterial flow and LDF signal is approximately linear, although at the highest bronchial arterial blood flow, i.e. 200% of control, the LDF signals tended to be slightly lower (176 $\pm$ 26%) than would be expected if a truly linear relationship held.

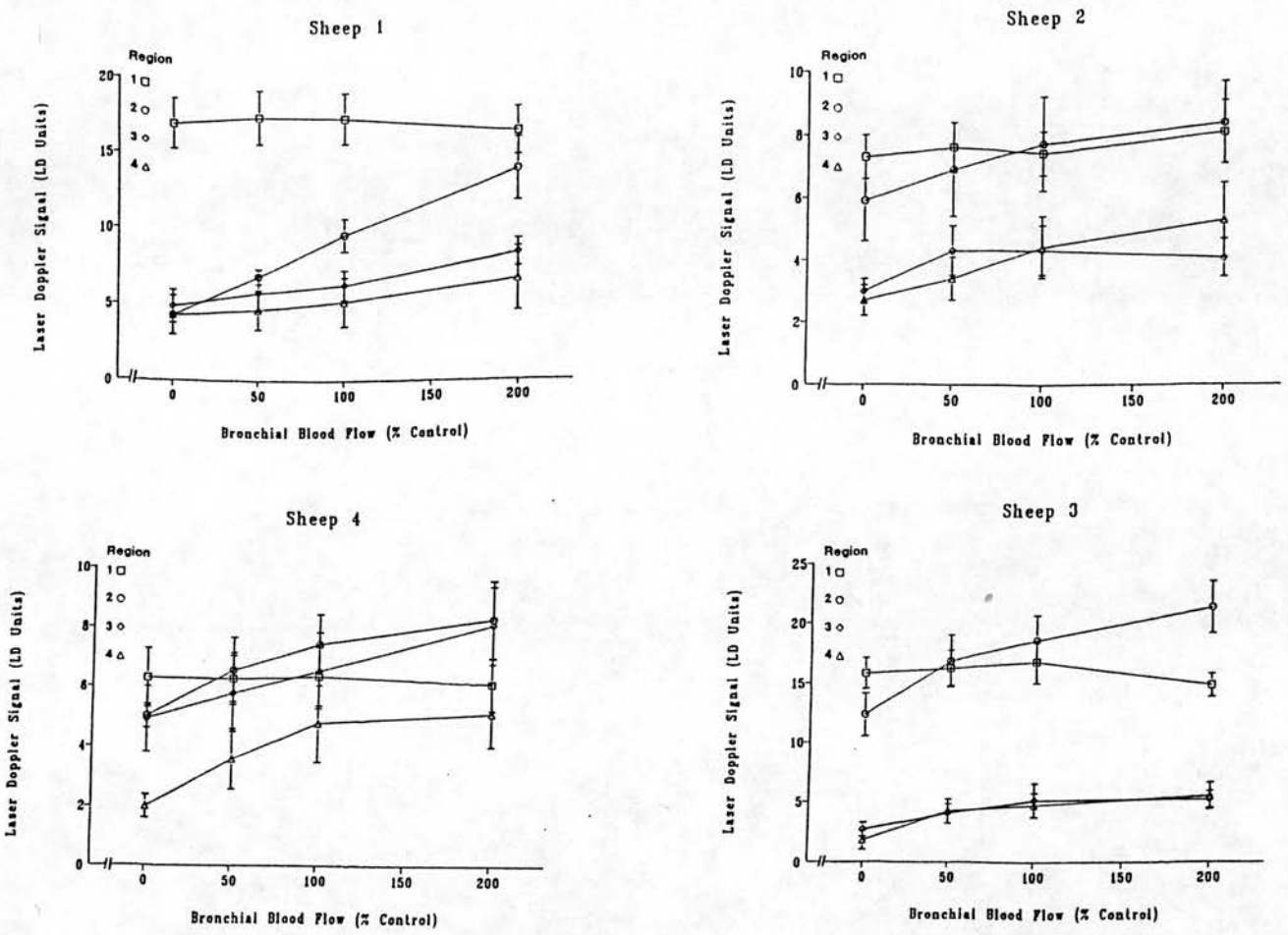


Figure 5.3. The LDF response to changing bronchial arterial blood flow in each region in each sheep. Data shown are untransformed and are expressed as mean (SE) for the ten sites studied.

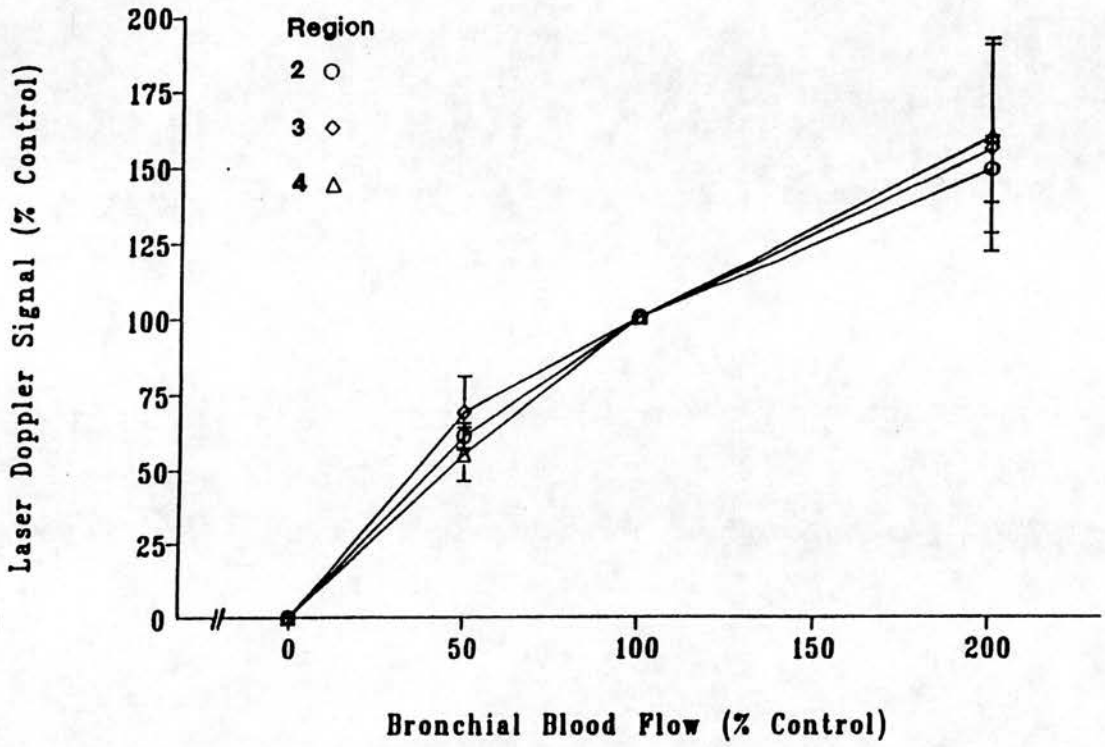


Figure 5.4. The LDF responses to changing bronchial arterial blood flow in regions 2, 3 and 4, after subtraction of the zero flow offset. LDF values (derived from raw data) are expressed as per cent of control.

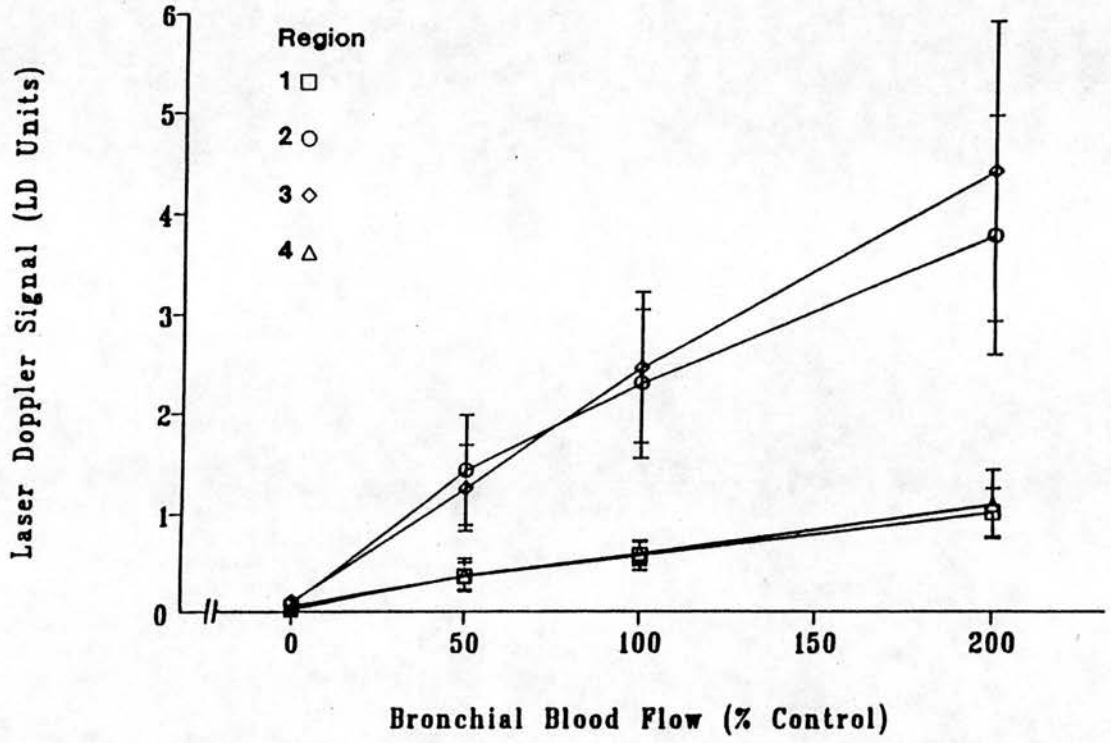


Figure 5.5. The LDF response to changing bronchial arterial blood flow after death. Data shown are untransformed and are expressed as mean (SE) for the ten sites studied.

The effects of perfusing the bronchial artery with a cell-free solution of dextran are shown for one sheep in figure 5.6. As the dextran solution washed into the bronchial arterial system, there was a gradual reduction in the LDF signal until a plateau was reached. A simultaneous fall (40 cm H_2O) in bronchial arterial pressure occurred, which was presumably caused by the lower viscosity of the dextran solution compared with blood. When whole blood was reintroduced into the bronchial arterial system, the LDF signal rose to almost its previous level. This procedure was repeated on four occasions in region 3 in two sheep, and the response was consistent. The LDF flow signal during dextran perfusion was, on average, 20 $\pm$ 12 % of that obtained during whole blood perfusion of the bronchial artery at the same flow rate.

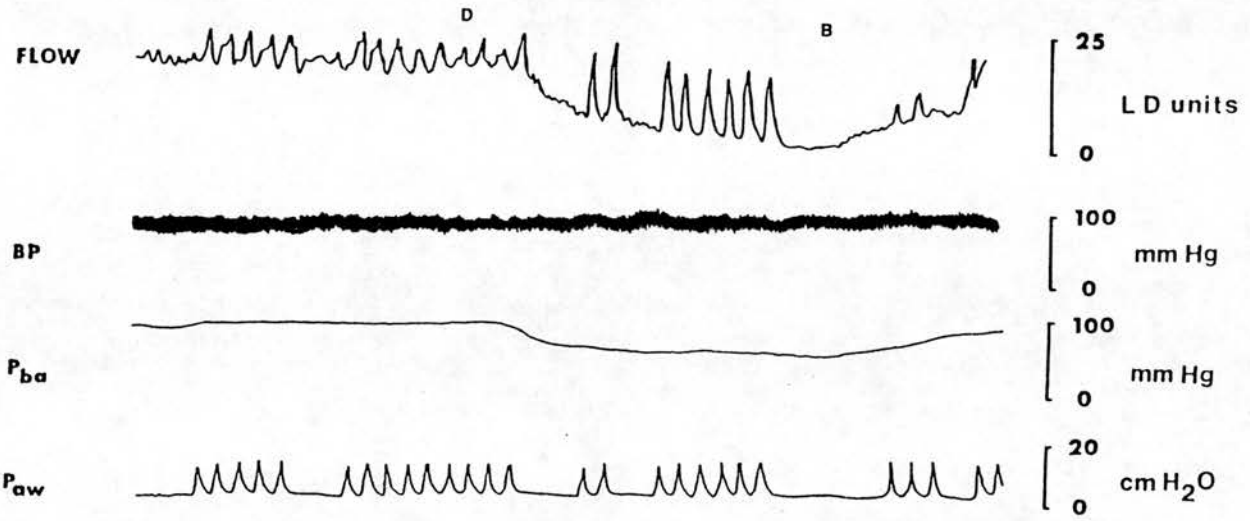


Figure 5.6. The effects, in one sheep, of perfusion of the bronchial artery with dextran solution. D indicates dextran perfusion, B indicates blood perfusion. BP, systemic arterial blood pressure, P_{ba}, bronchial arterial pressure; P_{aw}, airway pressure. Measurements were made during brief cessation of ventilation as indicated by the airway pressure signal. The delay in response when perfusion solution is changed is due to the wash-in period in the perfusion circuit.

5.7 DISCUSSION

In this study, it has been shown that non-invasive measurements of blood flow to the airway wall in sheep can be made using laser Doppler flowmetry, but detection of background noise is a problem in interpreting the LDF signals. LDF signals during the control bronchial flow rate showed considerable variation between sites, and between regions within individual sheep. This probably reflects the high spatial resolution of the LDF technique, and the non-homogeneous nature of the microvascular bed.

In the live sheep, varying the blood flow through the cannulated bronchial artery resulted in variations in LDF signals obtained in the principal bronchus, the lower lobe bronchus and the lower lobe segmental bronchi. However, there was no variation in LDF signal in the lower trachea, above the main carina. In the regions 2, 3, and 4 of the live sheep, the relationship between varying flow rates into the bronchial artery and the LDF signals was approximately linear, although at the highest level of bronchial arterial blood flow, the LDF signal tended to be lower than would be expected for a truly linear relationship. In the live sheep, there was a substantial residual LDF signal even when the bronchial arterial pump flow was set to zero.

In contrast, in the dead sheep, varying blood flow through the cannulated bronchial artery resulted in variations in LDF signals in the lower trachea as well as in the bronchi. This can be explained by the vascular anatomy and is discussed later. The relationship between the bronchial blood flow and the LDF signal was also approximately linear in all regions, although again at the highest flow level, the LDF signals tended to be slightly lower than would be expected. When flow into the bronchial artery was set to zero, only a very small LDF

signal persisted in the dead sheep. A reduced LDF signal was present when the bronchial artery was perfused with dextran solution. These observations suggest that, in addition to detecting a signal related to bronchial arterial blood flow, the LDF technique also detects collateral circulation to the region under study, or the presence of background "noise".

The variation in LDF signals between sites at the control blood flow is similar to that seen using LDF in other biological systems. In the skin, the coefficient of variation between sites for LDF measurements on the human forearm ranges from 23% to 75% (Johnson et al, 1984). This has been attributed to variation in capillary density in the skin which is approximately six-fold when $1mm^3$ areas of skin are compared (Landis, 1938). Similarly, in measuring gastric blood flow, a coefficient of variation of 33% to 39% has been demonstrated between sites (Kiel et al, 1985). In the present study, since the probe was held at each site until the protocol of varying bronchial blood flow was completed, this variation did not pose a problem. However, if the probe were to change sites during an intervention, this would be important. In these circumstances, if blood flow to a region were to be assessed, then multiple sites would have to be examined before and after the intervention, and average measurements compared.

The observed distribution of blood flow to regions of airway wall in which the LDF measurements were responsive to changes in the flow rate into the bronchial arterial system is consistent with the anatomical distribution of the systemic blood supply to the lungs. The major bronchi, in which changes in LDF flow signals were observed during changes in pump flow, are supplied by the cannulated vessel, the common bronchial branch of the bronchoesophageal artery. This

vessel, previously termed the carinal artery (Magno & Fishman, 1982), is known to provide the majority of the systemic blood supply to the lungs distal to the main carina (Magno & Fishman, 1982, Charan et al, 1987). The upper trachea, however, is supplied by a separate vessel, the tracheal bronchial artery, arising from the brachiocephalic trunk, and, in the lower trachea, an additional supply is derived from the thoracic tracheal branch of the bronchoesophageal trunk (Charan et al, 1987). Since the latter branch was tied off in this study, the blood supply to the lower trachea would be derived from the thoracic tracheal vessel, and therefore would not be influenced by changes in the pump flow. In contrast, the studies in the dead sheep show that, in the absence of perfusion from other sources, blood may flow from the bronchial arterial system into the distal trachea, indicating the presence of anastomoses between the bronchial and tracheal circulation. Flow probably does not occur through these vessels in the intact sheep, in which bronchial and tracheal vascular pressures would be similar. The results of this study also suggest that these anastomoses probably do not extend functionally above the level of the tracheal bronchus, since, in one sheep, in which measurements were made above the level of the tracheal bronchus after death, increasing the bronchial arterial blood flow to three times control levels did not produce any detectable LDF signal in the upper trachea. The data in figure 5.3 show an obvious decrease in LDF flow as one moves into the lung from site 2 to site 4. In two sheep (numbers 1 and 4), there is a progressive reduction in LDF flow at nearly all bronchial blood flows, whereas in the other two sheep, there is a decrease in LDF flow only between sites 2 and 3.

There are two possible reasons for the occurrence of an LDF signal when the bronchial arterial blood flow is zero. The first

could be the presence of collateral circulation to the airway wall, either from the other systemic vessels to the lung, or from the pulmonary circulation. In a recent study in dogs, Barman et al (1988) have shown that 50% of blood flow to the bronchial walls may be derived from the pulmonary circulation. The contribution of the pulmonary circulation to airway wall blood flow in sheep is not known. The second reason could be the detection of background "noise" by the LDF probe, which will detect any source of movement within its sampling volume. In studies of gastric mucosal blood flow, Kiel et al (1985) observed rhythmic oscillations in the LDF signal which corresponded to electrical activity in the gastric muscularis. Possible sources of noise in this study include tissue movement related to cardiac pulsation and smooth muscle contraction, ciliary movement, and Brownian motion of red cells within the microvascular bed (as discussed in chapter 3). These latter two sources may be the origin of the small signal persisting while the flow was set at zero after the death of the sheep. Small residual LDF signals have also been reported in other animals and other tissues after stopping the circulation (Stern et al, 1977, Ahn et al, 1987). Indeed, LDF has been used to study ciliary motion which is known to persist after cardiac arrest (Verdugo et al, 1988). In this study, these signals were of very small magnitude, ranging from 0.3% to 0.5% of the signal at control blood flow in the live sheep, and would therefore have only a minor influence on measurements in the intact animal.

In this study, we could not absolutely distinguish between "noise" and collateral circulation as possible causes of the residual LDF signal when flow was set to zero in the live sheep. However, the results of perfusion with dextran solution provide some insight into this problem. Since the principle of LDF is based on the detection of

movement of red blood cells, perfusion using a cell free solution shoud produce a flow signal of zero. In a study of canine gastric mucosal blood flow, Kiel et al (1987) found that during perfusion of an isolated gastric flap using a cell-free albumin solution, the laser Doppler flow signal fell almost to zero. In the present study, however, during dextran perfusion, the LDF signal was about 20% of the level observed during perfusion of whole blood. This suggests that this portion of the signal was related to motion of the living tissue elements in the sampling volume of the LDF probe. In contrast, when the bronchial arterial system was filled with blood, and the bronchial blood flow was set at zero, the LDF signal was about 60% of the level observed during control flow conditions. This higher signal, compared to that observed during dextran perfusion, could be due to the additional random motion of red cells in the vasculature within the sampling volume of the LDF probe, and also to the presence of collateral circulation. To resolve this question, a more complex preparation would be required, in which all potential sources of collateral circulation could be isolated and separately perfused.

The reponses of the LDF signal to changing bronchial arterial flows were approximately linear in regions 2, 3, and 4 in the live sheep, and in all four regions in the dead sheep. However, it is important to consider how the magnitude of changes in the LDF signals reflected the magnitude of change of pump flow into the bronchial arterial system. In the live sheep, when the actual LDF signals are expressed as percentages of the signal at control flow, then a 100% increase in bronchial arterial blood flow results in an increase in LDF signal of only 19 $\pm$ 16% in region 2 (principal bronchus), 15 $\pm$ 14% in region 3, and 16 $\pm$ 8% in region 4. Similarly, a 50% reduction in bronchial arterial blood flow results in a reduction of LDF signal of

only 15 ± 9% in region 2, 8 ± 7% in region 3, and 18 ± 7% in region 4. This apparent blunting of the LDF response is in part due to the offset from zero in the LDF signals. When the LDF signals at zero bronchial arterial flow are subtracted from the remainder of the LDF signals, and the new LDF values thus obtained are expressed as percentages of the values at control flow (figure 5.4), then the corresponding LDF values for a 100% icrease in bronchial arterial blood flow become 48 ± 22% in region 2, 50 ± 77% in region 3, and 60 ± 66% in region 4. For a 50% reduction in bronchial arterial blood flow, the corresponding reduction in LDF signal is now 40 ± 9% in region 2, 31 ± 24% in region 3, and 45 ± 18% in region 4. Thus the blunting of the LDF response is partly, but not entirely explained by the offset from zero.

Examination of responses at individual sites within a region indicated that the other major reason for the apparent blunting of overall response is variation between sites. Figure 5.7 shows the responses of ten sites in region 3 of one sheep, the LDF signals being expressed as percentages of the control flow signals. At some sites (e.g. 7 and 8), changes in bronchial arterial blood flow are associated with equivalent changes in LDF signal, whereas at other sites (e.g. 4 and 6), there is little or no change in the LDF signal across the range of blood flows. One explanation of this would be a failure of the LDF probe to detect changes at some sites. Alternatively, it is possible that blood flow at some sites is derived from another source such as the pulmonary circulation or collateral vessels. Finally, it is possible that the vasculature measured by the LDF probe at some sites may be capable of local autoregulation.

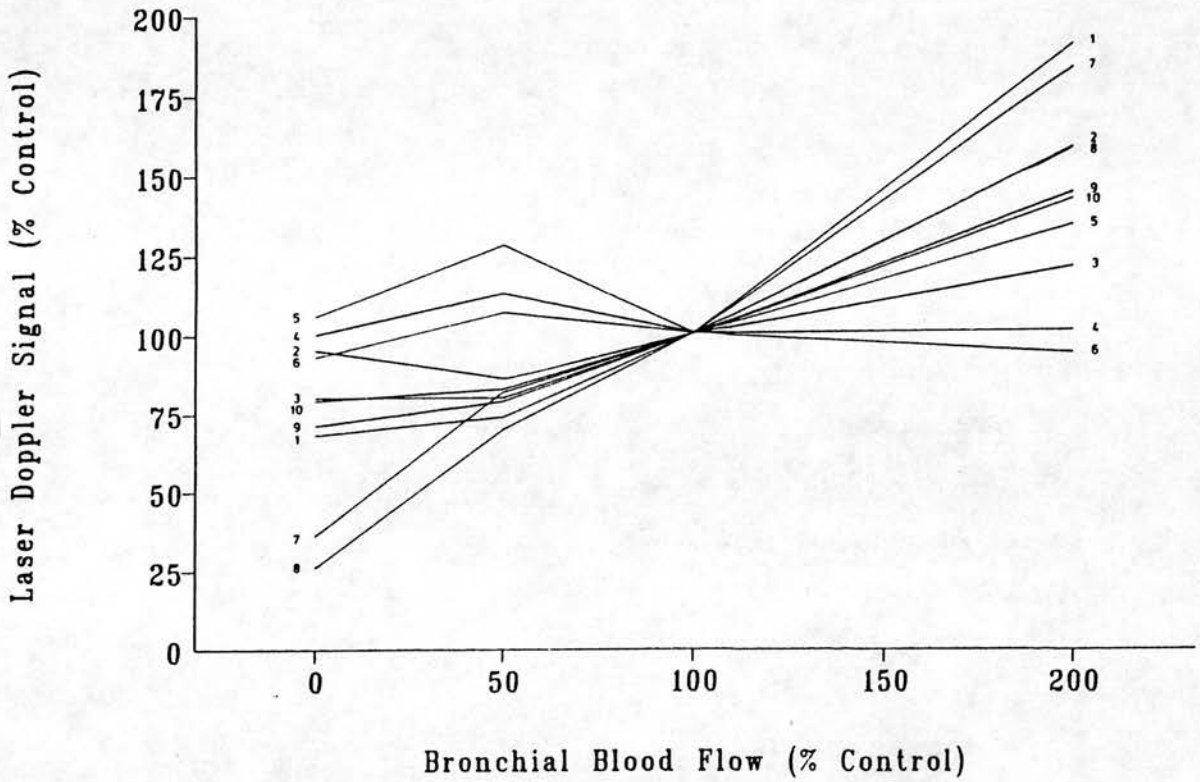


Figure 5.7. LDF responses to changing bronchial arterial blood flow at ten individual sites within one region. LDF values are expressed as per cent of control values.

In support of this latter suggestion is an observation made in one sheep, in which we examined the effect of an infusion of papaverine, a smooth muscle relaxant and vasodilator (Goodman & Gilman, 1985), into the bronchial artery. As shown in figure 5.8, recordings at one site within region 3 in this sheep initially showed no response of the LDF signal to changing bronchial arterial blood flow. While maintaining the LDF probe at the same site, an infusion of 30mg of papaverine was given over 20 seconds into the bronchial artery, and 45 seconds later, the protocol of varying bronchial arterial blood flow was repeated. There was now a clear response of the LDF signals to varying blood flow. The sheep was then killed with an overdose of intravenous sodium pentobarbital, and the protocol was repeated. The LDF signal remained responsive to varying bronchial arterial blood flow, and the zero flow offset was now very small. Although this example suggests that autoregulation of blood flow may be occurring, further studies are necessary to examine this question fully.

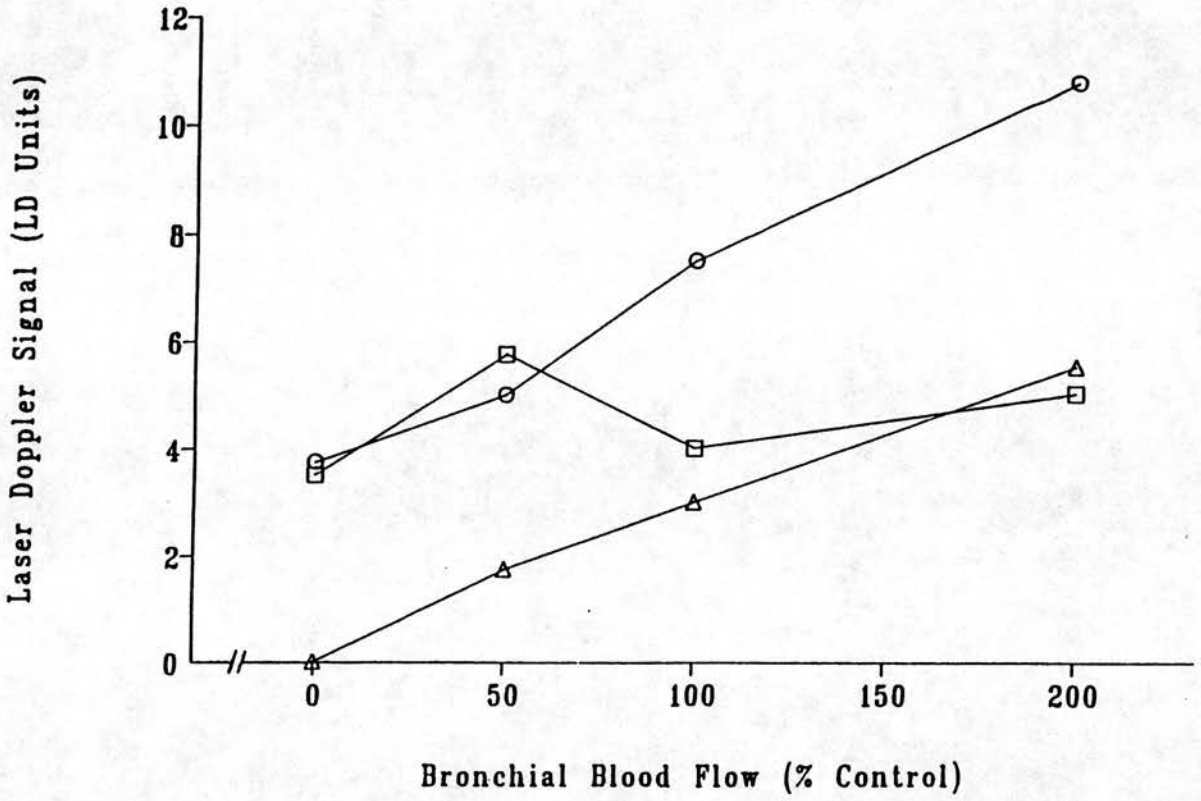


Figure 5.8. LDF responses to changing bronchial arterial blood flow at one site in the left principal bronchus of one sheep before □ , and after ○ an infusion of papaverine, and after death △ .

Laser Doppler flowmetry has been used in two previous studies to assess airway wall blood flow (Lin et al, 1987, Martling et al, 1987). In one of these studies, the probe was inserted surgically through the tracheal wall of cats (Martling et al, 1987). In the other study in sheep (Lin et al, 1987), published only in abstract form, the probe was held in place mechanically and a number of technical limitations were encountered, particularly problems related to mechanically induced airway hyperaemia. In the latter study, a comparison with radioactive microsphere measurements was attempted, but a universal calibration factor could not be identified. In both studies, anaesthesia and extensive surgical preparation ventilation were necessary. In the present study, it has been shown that measurements of airway wall blood flow may be made using LDF during bronchoscopy. The technique is non-invasive and provides continuous, real-time measurements. When measurements from ten sites are averaged, then the LDF signal measured over a range of bronchial blood flow from 1.2 ml/kg body weight/min to zero is approximately linear, although considerable site-to-site variation in response occurs. Interpretation of the LDF signals is complicated by the effects of background "noise" on the signal, and since the sampling volume of the LDF probe in the airways remains unknown, calibration of the signal in units of blood flow per unit mass of tissue has not been achieved. The LDF system may, however, have potential for studying changes in airway wall blood flow in spontaneously breathing subjects. The next chapter describes an experiment designed to permit a comparison of LDF measurements of airway blood flow with measurements obtained using an established method of measuring microcirculatory flow, the radiolabelled microsphere reference flow technique.

CHAPTER 6

A COMPARISON OF LASER DOPPLER FLOWMETRY AND THE REFERENCE FLOW TECHNIQUE TO MEASURE TRACHEAL WALL BLOOD FLOW IN DOGS

6.1 SUMMARY

Measurements of tracheal wall blood flow were made in anaesthetised, ventilated dogs using laser Doppler flowmetry and the radiolabelled microsphere reference flow technique during resting ventilatory conditions, during application of 15 cm H2O PEEP, and during eucapnic dry air hyperventilation. Six regions of the trachea were studied in each dog, and the average LDF measurement derived from 12 sites within each region was compared with the reference flow measurement, obtained using 15 micron diameter microspheres labelled with ^{113}Sn, ^{103}Ru, ^{103}Gd or ^{141}Ce. Under each set of ventilatory conditions, there was a weak, but significant correlation between LDF and reference flow values (baseline $r^2 = 0.19$, hyperventilation $r^2 = 0.19$, and PEEP, $r^2 = 0.54$). Both LDF and reference flow values showed reductions from baseline during application of PEEP. The mean (SD) change from baseline, expressed as a percentage, was -63 (15)% for LDF values, and -63 (21)% for reference flow values. There was, however, no correlation between LDF and reference flow values for individual sites. During hyperventilation, LDF measurements showed variable changes from baseline. The mean change was -12 (45) %, which was not significantly different from zero. Reference flow values increased during hyperventilation, the mean change being 87 (77)%. Further analysis of reference flow data indicated that changes in blood flow were not uniform throughout the tracheal wall. The study indicates that LDF and reference flow measurements of airway wall blood flow are not directly comparable.

6.2 INTRODUCTION

In the study described in the previous chapter, total bronchial arterial blood flow was varied while laser Doppler measurements of airway blood flow were obtained. Direct comparison of the flow measurements was therefore complicated by possible local variations in microcirculatory blood flow e.g. the occurrence of local autoregulation or of vascular "steal" phenomena. This study was designed to provide a direct comparison between laser Doppler flow measurements and an established method of measuring microcirculatory blood flow, the radiolabelled microsphere reference flow technique. Tracheal wall blood flow was measured in several regions of the trachea of dogs using both the laser Doppler flow technique and the radiolabelled microsphere reference flow technique. An assessment was made of the effects on these measurements of two separate stimuli: hyperventilation of warm dry air, which is known to increase tracheal and bronchial blood flow in animals (Baile et al, 1987 a and b, Parsons et al, 1989), and application of positive end-expiratory pressure (PEEP), which is known to reduce tracheal blood flow (Baile et al, 1984).

6.3 METHODS

6.3.1 Preparation of animals

Five mixed breed dogs of mean weight 31±6 kg were studied. All were anaesthetised with intravenous pentobarbital sodium, 25 mg/kg, tracheostomised, and studied in the supine position. Anaesthesia was maintained by supplemental doses of pentobarbital, paralysis was maintained using intravenous pancuronium bromide, 2mg, prior to obtaining each set of LDF measurements, and atropine was administered

(0.6 mg IV at the beginning of the study, followed by supplemental doses of 0.4 mg prior to obtaining each set of LDF measurements) to prevent excess secretions. The dogs were ventilated using a Harvard animal respirator set to provide a tidal volume of 15 ml/kg and a rate of 15 breaths/min. Inspired air was warmed by passing it through a series of copper coils in a water bath, and humidified using a ultrasonic nebuliser (Devilbiss 65, setting 1-2). End tidal carbon dioxide tension was measured using an infra-red CO_2 analyser (Hewlett Packard, Capnometer, model 47210A). A thermistor tipped, triple-lumen catheter was inserted, under fluoroscopic control, into the pulmonary artery for measurement of pulmonary arterial pressure and of cardiac output by the thermodilution technique. Catheters were also inserted into the left ventricle for injection of radiolabelled microspheres, the aorta for measurement of systemic arterial blood pressure, and the femoral arteries and veins for blood sampling and drug administration. All pressures were referenced to the level of the left atrium.

An Olympus IT 10 bronchoscope was then inserted through the tracheostomy tube and the distance from the tip of the tracheostomy tube to the main carina was measured in each dog. By dividing this distance into three equal portions, and studying the left and right lateral tracheal walls separately, six regions of the trachea were defined in which the LDF measurements were obtained, namely upper right and left, middle right and left, and lower right and left trachea (figure 6.1).

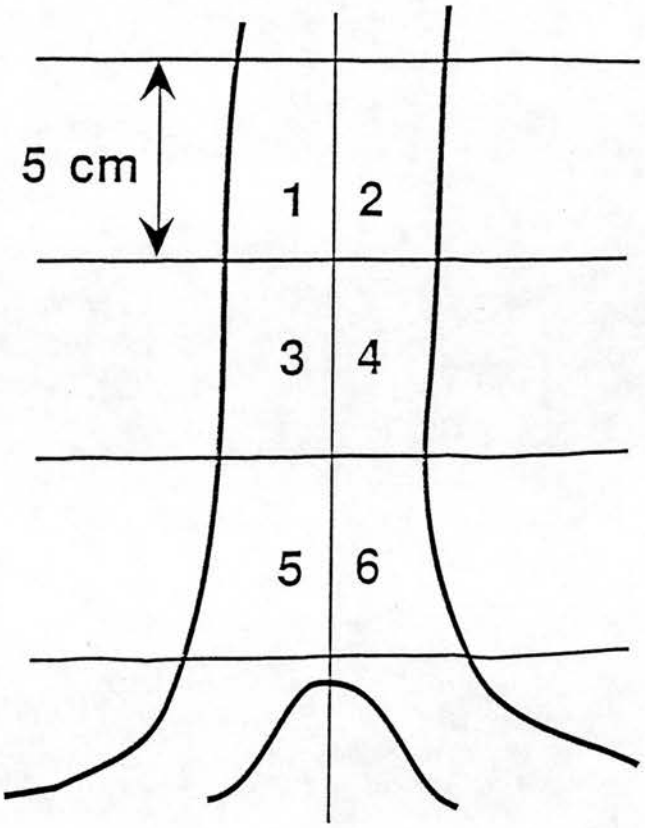


Figure 6.1 (a). The regions of the trachea in which measurements were made are indicated.

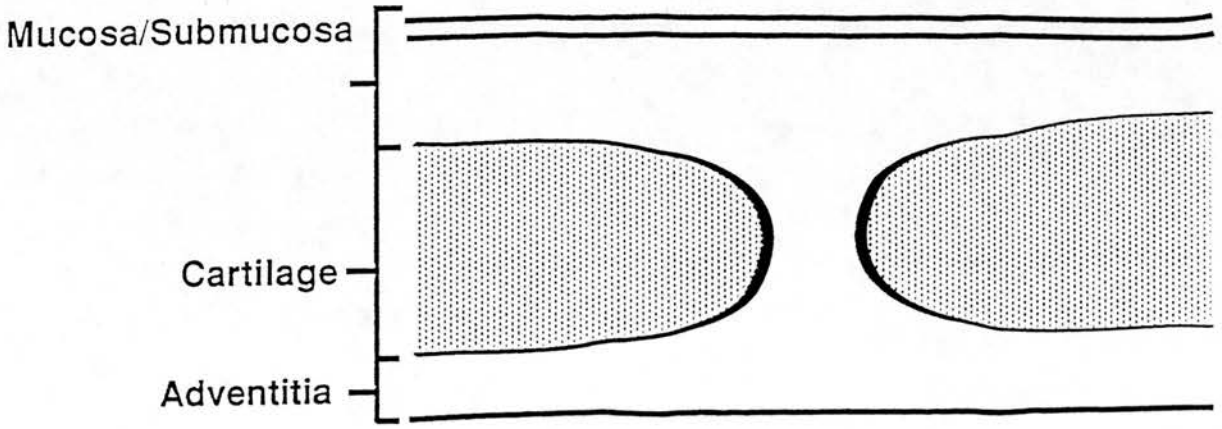


Figure 6.1 (b). The levels of the tracheal wall at which reference flow measurements were obtained are indicated.

6.3.2. Study Protocol

The study protocol was as follows. LDF measurements were made, as described later, at 6 sites within each region of the trachea during baseline ventilation (V_t 15 ml/kg, rate 15/min). After this a reference flow measurement was obtained as described later, and the LDF measurements were then repeated. Each dog was then hyperventilated with warm dry (cylinder) air (V_t 25 ml/kg, rate 40/min), and the protocol of LDF and reference flow measurements was repeated. During this period, carbon dioxide was added to the inspired gas to maintain eucapnic conditions. During a third period, the dogs were again ventilated with warmed, humidified air (V_t 20 ml/kg, rate 15/min) while 15 cmH_2O PEEP was applied, and the protocol of LDF and reference flow measurements was repeated. The higher tidal volume during the third period was necessary in view of the increased deadspace associated with the application of PEEP.

6.3.3 Technique of Laser Doppler Flow Measurements

The method of obtaining LDF measurements was again as described in chapter 2. The laser Dopppler flow probe was introduced through the suction channel of the bronchoscope, and was advanced under direct vision to about 2-3 cm distal to the tip of the bronchoscope. The tip of the probe was allowed to rest lightly on the mucosa, and, during each measurement, ventilation was stopped briefly (about 10 sec) to prevent ventilator motion artifact. Flow, volume and velocity signals were continuously displayed on an 8-channel recorder. The average flow signal at each site during each recording period was determined visually from the recording (figure 6.2).

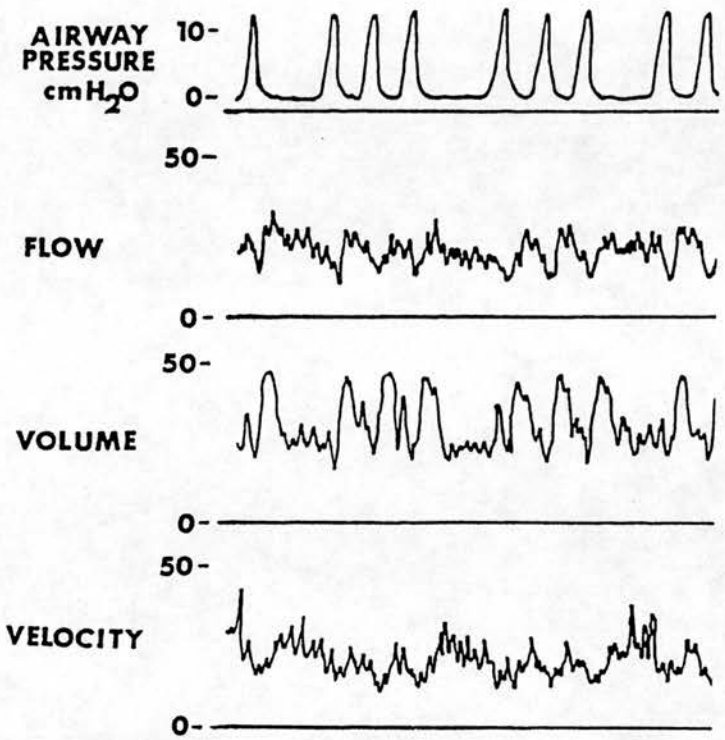


Figure 6.2. Specimen tracing showing 3 periods of cessation of ventilation (indicated by airway pressure of 0 cm H2O). The corresponding LDF flow, volume and velocity signals are shown in the lower panels. Ventilation artifact is most obvious in the volume signal.

Six sites were studied in each region, with five breaths being allowed between each LDF measurement. The light intensity of the fibreoptic bronchoscope was reduced to a low level to prevent interference with the LDF signal. For the LDF measurements, the mean of the 12 measurements made in each region during any set of ventilatory conditions was taken as the representative value for that region under those ventilatory conditions.

6.3.4 REFERENCE FLOW TECHNIQUE

The radiolabelled microsphere measurements of blood flow were obtained using the reference flow method developed by Baile et al (1982), which is a modification of the technique described by Rudolph and Heymann (1967). Before making each reference flow injection, measurements of vascular pressures, cardiac output, arterial blood gases, inspired air and body temperature were made. Tracheal blood flow was then determined by the injection of about 3 MegaBecquerels (37 MBq = 1 mCi), 15 micron diameter radiolabelled microspheres into the left ventricle. ^{113}Sn, ^{103}Ru, and ^{103}Gd or ^{141}Ce-labelled microspheres supended in 0.9% saline with 0.01% Tween 80 (NEN-TRAC microspheres, DuPont) were used in random order to make measurements of blood flow under each set of ventilatory conditions.

The protocol for the reference flow measurements was as follows: a femoral arterial reference flow sample was begun at time zero and blood was withdrawn over a two minute period using a withdrawal pump (Harvard) at a rate of 10ml/min. Ventilation was stopped at this time for a period of 30 seconds. Five seconds after starting the pump, microspheres, suspended in 5 ml saline, were injected into the left ventricle over 5-10 seconds. At two minutes, the withdrawal pump was stopped.

After the final measurement of blood flow, each dog was killed by an overdose of sodium pentobarbital and exsanguination. The trachea was removed and sectioned into six regions corresponding to those in which the LDF measurements had been made. The mucosa was stripped from the underlying cartilage, and the adventitial layer was also removed. The blood and tissue samples were weighed and the radioactivity in the reference flow blood samples and in the tissue samples was measured in a Beckman 8000 scintillation counter linked to a Nucleus PCA analyser (Nucleus, Oak Ridge TN) operating as a pulse height analyser. This analyser, by means of a computer program employing a multiple linear regression analysis, allows measurement of the activity of each of the isotopes, while correcting for decay and providing an estimate of counting error for each isotope. Each sample was counted over a 2-minute period. Blood flow to the mucosa, cartilage and adventitial layer was determined for each region under each set of ventilatory conditions.

Blood flow was calculated as previously described by Baile et al (1982), by applying a proportional model in which, when radioactive counts in the tissue samples and in the reference flow blood sample are known, and the reference flow rate is known, then blood flow to the tissue sample can be determined.

$$\text{Blood flow per tissue sample} = (At/Af)*(Qref)$$

where At and Af are the radioactive counts in the tracheal tissue and the reference blood flow samples respectively, and Qref is the reference flow rate in ml/min.

In practice, the reference flow blood sample for each isotope was dispensed into 4 vials each containing 4-5 ml of blood, and radioactive counts for each sample were determined separately. Blood

and tissue vial weights, empty and full, together with radioactive counts were then entered into a spreadsheet (Lotus 1-2-3, Lotus Development Corp., Cambridge., MA), an example of which is shown in figure 6.3. Using this spreadsheet, the mean radioactive counts per gram of blood were determined for each of the reference flow samples. A flow factor was then computed for each isotope as follows:

$$\text{FLOW FACTOR} = \frac{(\text{REFERENCE FLOW RATE/REFERENCE FLOW VOLUME})}{\text{COUNTS PER GRAM}}$$

$$= \frac{(10\text{ml/min}) / (20\text{ ml})}{\text{COUNTS PER GRAM}}$$

$$= \frac{0.5}{\text{COUNTS PER GRAM}}$$

The product of this flow factor and the radioactive counts from any tissue sample then represents the blood flow to that sample in ml/minute. From a knowledge of the cardiac output, measured in this study by the thermodilution method, the blood flow to the tissue sample can be expressed as a percentage of cardiac output. Blood flow can also be expressed per unit mass of tissue, since the weight of each tissue sample is known.

Date : JUN589
Exper. : LDF2

Vial Number	Vial Weight	Vial Wet Wt.	Wet Wt. (gm)	cts/gm One	cts/gm Two	cts/gm Three	cts/gm Four	Original Count Indicators I	II	III	IV
1	13.290	18.000	4.710	5733.1				27004	668	375	
2	13.294	16.722	3.428	5192.8				17803	328	197	
3	12.805	16.190	3.385	6328.5				21424	340	222	
4	12.790	15.887	3.098	6185.4				19160	230	108	
5	13.377	17.490	4.112		4673.4			0	19219	341	
6	12.840	17.019	4.179		4595.0			0	19202	284	
7	13.349	17.582	4.233		4438.0			0	18786	330	
8	12.696	16.975	4.279		4578.8			0	19591	360	
9	13.348	17.500	4.152			10203.3		67	1108	42361	
10	13.315	17.416	4.101			11107.8		41	846	45552	
11	12.835	17.008	4.173			13482.9		157	969	56267	
12	13.309	17.571	4.263			10448.1		59	769	44537	
13	13.287	0.000	-13.287				0.0	6	22	21	
14	12.816	16.348	3.532				0.0	3	21	26	
15	13.330	16.826	3.496				0.0	2	16	27	
16	13.356	16.880	3.524				0.0	4	21	21	
17	12.785	13.254	0.470	----------				1939	5117	2356	
18	13.328	13.597	0.269	Mean counts per gram				1298	2018	846	
19	13.302	13.677	0.374	5860.0	4571.3	11310.5	0.0	554	1285	719	
20	13.282	13.893	0.612	----------				458	580	285	
21	12.792	16.776	3.984	Flow Factors				7	27	20	
22	13.220	14.029	0.809	0.000085	0.000109	0.000044	ERR	693	407	295	
23	13.340	13.564	0.224	----------				6400	8301	5619	
24	12.773	12.981	0.208					6129	8824	2731	
25	12.802	14.330	1.528					2432	1213	773	
26	12.862	14.195	1.332					553	335	328	
27	12.828	13.176	0.348					516	200	95	
28	12.831	13.130	0.298					205	80	229	
29	12.834	13.041	0.207					5618	8552	5533	
30	12.806	13.018	0.212					1568	5039	3673	
31	12.802	14.020	1.218					380	1267	814	
32	13.302	14.490	1.189					285	456	411	
33	13.299	13.528	0.230					47	215	99	
34	12.808	13.187	0.379					106	570	187	
35	12.835	13.007	0.173					4857	5920	2409	
36	12.758	12.895	0.138					2289	4919	3215	
37	12.762	13.958	1.197					1146	1916	912	
38	12.773	13.831	1.058					569	303	975	
39	12.906		-12.906								
40	13.344		-13.344								

Figure 6.3 (a) An example of the spreadsheet used to calculate blood flow. This indicates weights and radioactive counts in each blood and tissue sample.

Cardiac Outputs

4.7 4.84 3.03 0

Vial Number	Flow (ml/minute) I	II	III	IV	Percent Cardiac Output I	II	III	IV	ml/min/100gm I	II	III	IV
17	0.1654	0.5597	0.1042	ERR	0.0035	0.0116	0.0034	ERR	35.23	119.18	22.18	ERR
18	0.1108	0.2207	0.0374	ERR	0.0024	0.0046	0.0012	ERR	41.20	82.11	13.91	ERR
19	0.0473	0.1406	0.0318	ERR	0.0010	0.0029	0.0010	ERR	12.63	37.54	8.49	ERR
20	0.0391	0.0634	0.0126	ERR	0.0008	0.0013	0.0004	ERR	6.39	10.37	2.06	ERR
21	0.0006	0.0030	0.0009	ERR	0.0000	0.0001	0.0000	ERR	0.01	0.07	0.02	ERR
22	0.0591	0.0445	0.0130	ERR	0.0013	0.0009	0.0004	ERR	7.31	5.50	1.61	ERR
23	0.5461	0.9079	0.2484	ERR	0.0116	0.0188	0.0082	ERR	243.68	405.15	110.84	ERR
24	0.5230	0.9652	0.1207	ERR	0.0111	0.0199	0.0040	ERR	251.42	464.01	58.04	ERR
25	0.2075	0.1327	0.0342	ERR	0.0044	0.0027	0.0011	ERR	13.58	8.68	2.24	ERR
26	0.0472	0.0366	0.0145	ERR	0.0010	0.0008	0.0005	ERR	3.54	2.75	1.09	ERR
27	0.0440	0.0219	0.0042	ERR	0.0009	0.0005	0.0001	ERR	12.66	6.29	1.21	ERR
28	0.0175	0.0088	0.0101	ERR	0.0004	0.0002	0.0003	ERR	5.86	2.93	3.39	ERR
29	0.4794	0.9354	0.2446	ERR	0.0102	0.0193	0.0081	ERR	231.01	450.79	117.88	ERR
30	0.1338	0.5512	0.1624	ERR	0.0028	0.0114	0.0054	ERR	63.05	259.73	76.52	ERR
31	0.0324	0.1386	0.0360	ERR	0.0007	0.0029	0.0012	ERR	2.66	11.38	2.95	ERR
32	0.0243	0.0499	0.0182	ERR	0.0005	0.0010	0.0006	ERR	2.05	4.20	1.53	ERR
33	0.0040	0.0235	0.0044	ERR	0.0001	0.0005	0.0001	ERR	1.75	10.24	1.91	ERR
34	0.0090	0.0623	0.0083	ERR	0.0002	0.0013	0.0003	ERR	2.39	16.47	2.18	ERR
35	0.4144	0.6475	0.1065	ERR	0.0088	0.0134	0.0035	ERR	240.24	375.37	61.74	ERR
36	0.1953	0.5380	0.1421	ERR	0.0042	0.0111	0.0047	ERR	141.84	390.73	103.21	ERR
37	0.0978	0.2096	0.0403	ERR	0.0021	0.0043	0.0013	ERR	8.17	17.51	3.37	ERR
38	0.0485	0.0331	0.0431	ERR	0.0010	0.0007	0.0014	ERR	4.59	3.13	4.07	ERR
39	0.0000	0.0000	0.0000	ERR	0.0000	0.0000	0.0000	ERR	0.00	0.00	0.00	ERR
40	0.0000	0.0000	0.0000	ERR	0.0000	0.0000	0.0000	ERR	0.00	0.00	0.00	ERR
41	0.0000	0.0000	0.0000	ERR	0.0000	0.0000	0.0000	ERR	0.00	0.00	0.00	ERR
42	0.0000	0.0000	0.0000	ERR	0.0000	0.0000	0.0000	ERR	0.00	0.00	0.00	ERR

Figure 6.3 (b) This portion of the spreadsheet shows blood flow values calculated from the data in (a). Flow is expressed in absolute values(ml/minute), per unit mass of tissue (ml/min/100g), and as a percentage of cardiac output. The spreadsheet can deal with four separate isotopes, but in this study, only 3 were used.

By summing the weights and radioactive counts for each of the three levels of the tracheal wall in each region, a figure for blood flow to the total thickness of the tracheal wall in each region was obtained. This figure was used for comparison with the LDF measurements. In addition, the weights of tissue samples and radioactive counts for the whole trachea of each dog were summed, allowing calculation of an overall figure for tracheal blood flow in each dog during each set of ventilatory conditions. This measurement was compared with the average LDF value obtained in each dog. Blood flow measurements determined by the radiolabelled microsphere reference flow technique will be subsequently described as reference flow measurements. LDF and reference flow measurements were compared by linear regression analysis using the method of least squares (Zar, 1984).

6.4 RESULTS

Haemodynamic, arterial blood gas and temperature measurements are summarised in table 6.1. Arterial P_{co2} was slightly higher during the baseline period than during the subsequent periods, and inspired temperature was higher during the hyperventilation period. There was a significant fall in cardiac output during the application of PEEP, to an average of 53% of the baseline value. No significant changes in the other parameters occurred during the protocol.

TABLE 6.1 HAEMODYNAMIC, ARTERIAL BLOOD GAS and TEMPERATURE MEASUREMENTS DURING DIFFERENT VENTILATORY CONDITIONS

	Baseline	Hyperventilation	PEEP
BP	236 (29)	221 (31)	193 (34)
P_{pa}	26 (7)	29 (7)	36 (6)
P_{ra}	6 (2)	7 (4)	12 (5)
pH	7.3 (.03)	7.34 (.07)	7.3 (.1)
pO_2	145 (20)	115 (13)	144 (42)
pCO_2	42 (3)	35 (4)	32 (8)*
T_i	31 (2)	37 (3)*	32 (4)
T_b	38 (1)	38 (1)	38 (2)
CO	3.9 (.53)	3.4 (.9)	2.1 (.9)**

BP, systemic arterial pressure, cm H_2O; P_{pa}, pulmonary arterial pressure, cm H_2O; P_{ra}, right atrial pressure, cm H_2O; pO_2, pCO_2, mm Hg; T_i, inspired temperature, °C; T_b, body temperature, °C; CO, Cardiac output, L/min. Values are mean (SD). Difference from baseline significant at * 5% and **1% level.

There was considerable site-to-site variation in the LDF measurements within each region, the mean (SD) coefficient of variation (CV) between sites being 53 $\pm$ 19 % (range 17 -99 %). The variation was similar during all ventilatory conditions with values of 56 % (range 25 - 99 %) during hyperventilation and 51 % (range 17 - 89%) during PEEP). Variation in blood flow between regions was similar for both LDF and reference flow measurements. Under baseline ventilatory conditions, the CV between regions within dogs was 35 $\pm$ 8% for the LDF measurements, and 35 $\pm$ 15% for the reference flow measurements.

Figure 6.4 a, b and c show linear regression plots comparing LDF with reference flow values for the full thickness of the tracheal wall in each region during each ventilatory condition. The correlation between the LDF values and the reference flow values was significant but weak, particularly during baseline conditions and hyperventilation, and was highest during application of PEEP (baseline conditions, r^2=0.19, $p<0.02$; hyperventilation, r^2=0.19, $p<0.02$; PEEP, r^2=0.54, $p<0.001$). A single value for blood flow to the whole trachea under each ventilatory condition was calculated for each technique, and a comparison of the resulting flow values is shown in figure 6.5. There was again a rather weak though significant correlation (r^2=0.50, $p<0.01$) between LDF and reference flow measurements.

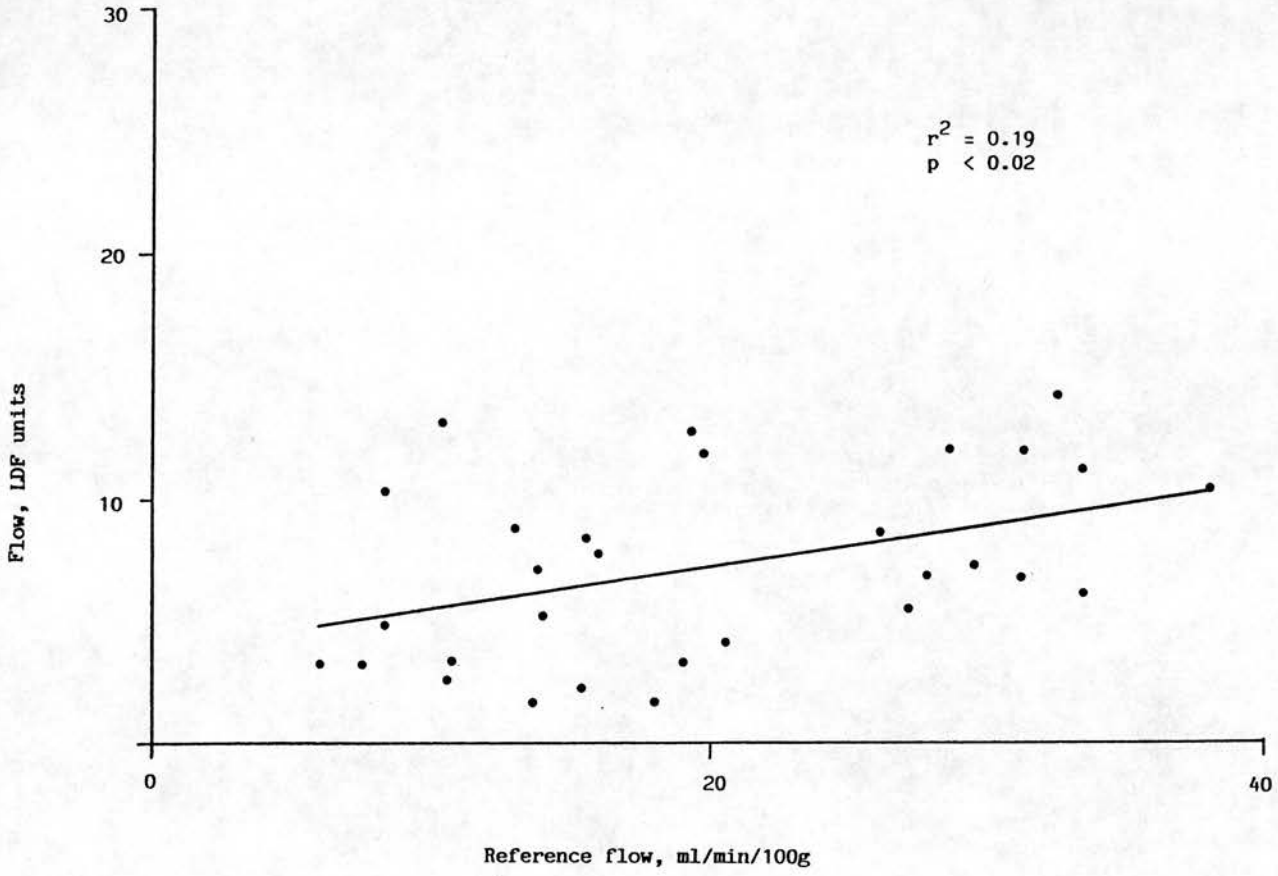


Figure 6.4 (a). Regression analysis of LDF versus reference flow values for blood flow in all regions of all dogs, during baseline ventilatory conditions. (y = 3.71 + 0.175 x).

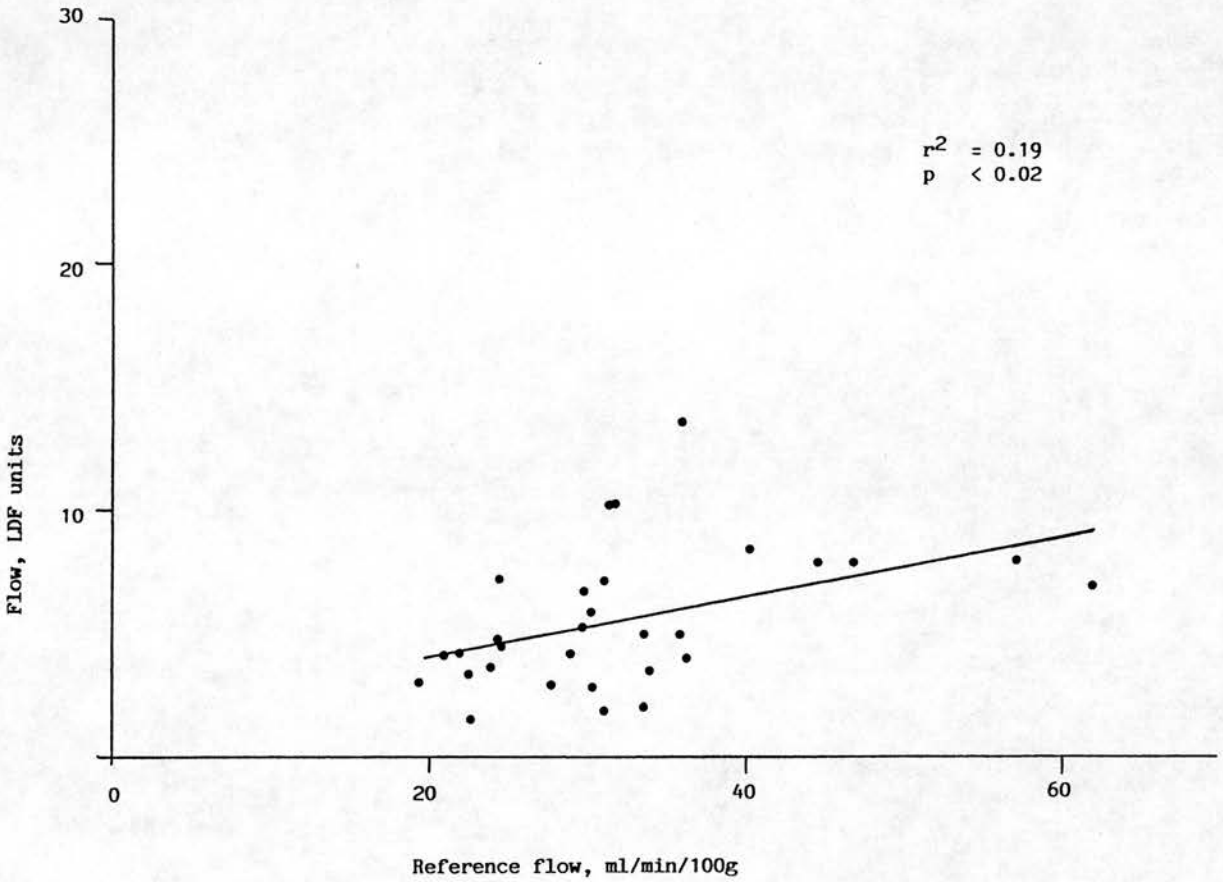


Figure 6.4 (b). Regression analysis of LDF versus reference flow values for blood flow in all regions of all dogs, during hyperventilation. ($y = 1.568 + 0.124\ x$)

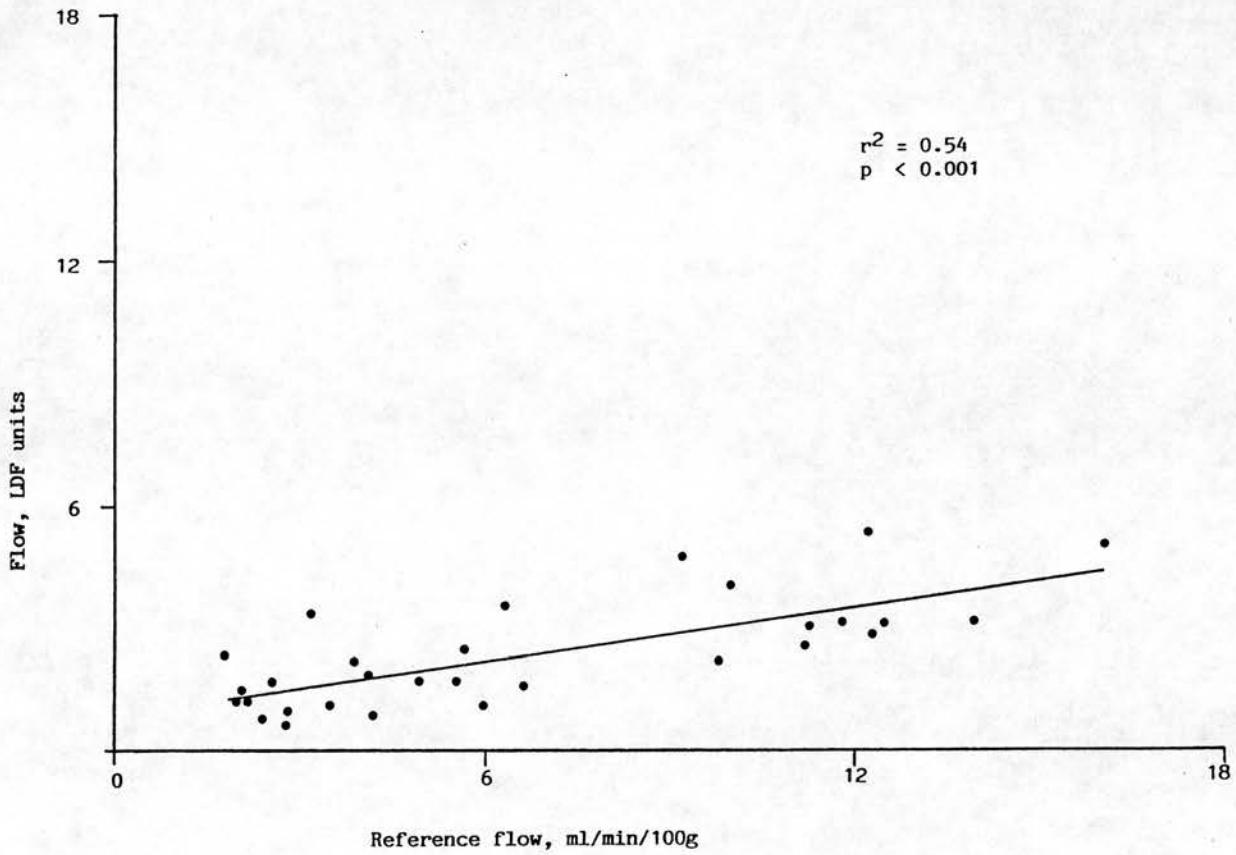


Figure 6.4 (c). Regression analysis of LDF versus reference flow values for blood flow in all regions of all dogs, during application of PEEP. (y = 0.903 + 0.215 x)

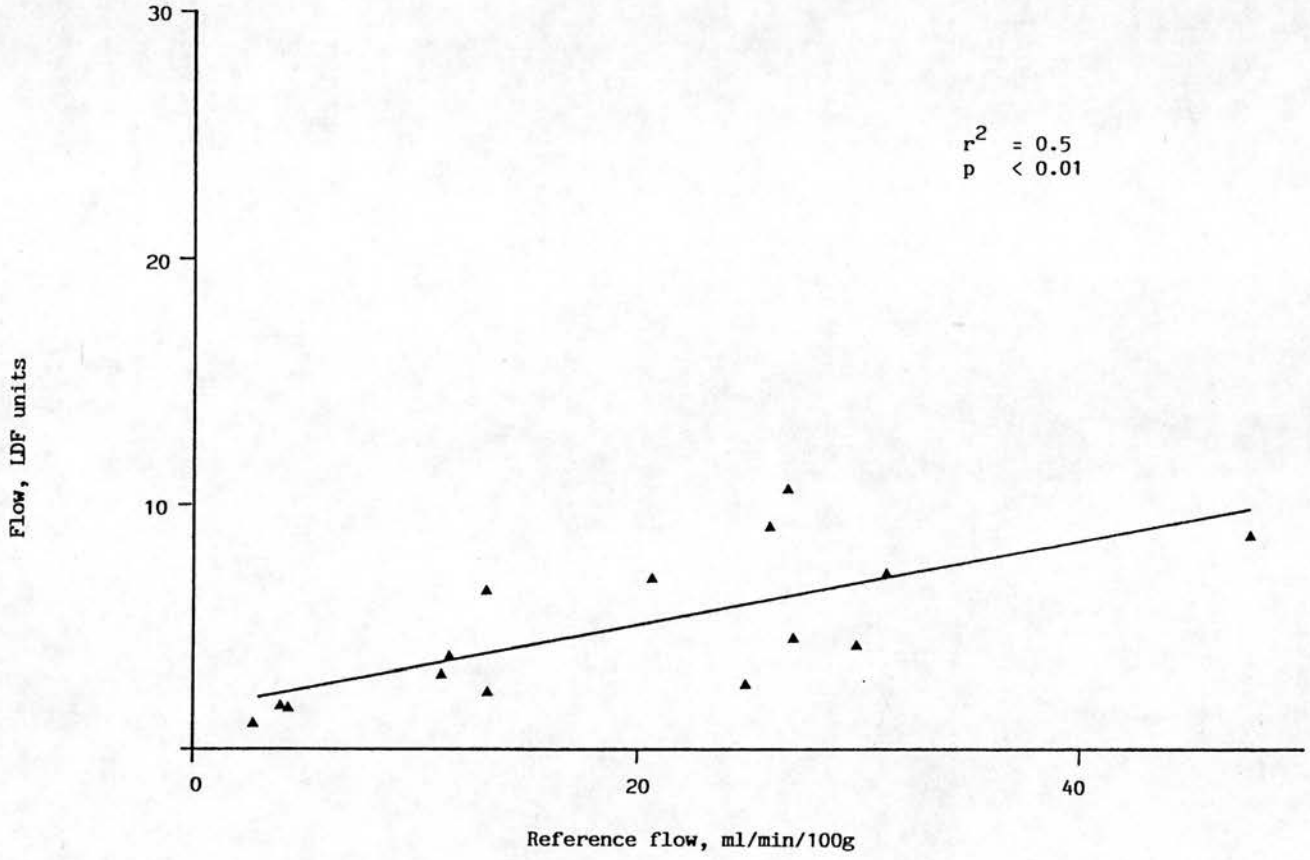


Figure 6.5. Regression analysis of LDF versus reference flow values for the whole trachea of each dog during all ventilatory conditions. (y = 1.64 + 0.173 x)

When the changes from baseline blood flow in each region during hyperventilation and application of PEEP were examined, major differences were observed between the techniques. The effects of hyperventilation of dry air are shown in figure 6.6. Reference blood flow values showed an increase during hyperventilation in all except five regions, the mean $\pm$ SD increase being 89 $\pm$ 77 %. However, the LDF values were much more variable, and the mean change (-12 $\pm$ 34 %) was not significantly different from zero. During PEEP, reference blood flow values showed a decrease in blood flow in all regions of the trachea, the mean decrease from baseline being 63 $\pm$ 21%. LDF values also decreased in all except one region, the mean decrease from baseline being 63 $\pm$ 15 %. However, when changes in individual regions were examined, there was no correlation between the reference flow and LDF measurements (r^2 = 0.02, p = NS)(figure 6.7).

Closer analysis of the reference flow data, however, revealed that the blood flow responses to hyperventilation of dry air were not uniform throughout the full thickness of the airway wall. Figure 6.8 shows the blood flow response to hyperventilation in each of the three levels of the airway wall (mucosa, cartilage and adventitia). In the mucosa, there was a rise in blood flow in almost all regions, the mean increase being 166 ($\pm$177) %. In the cartilage and the adventitia, however, the responses were much more variable, and the mean changes, +6 ($\pm$77)% and +41 ($\pm$161) % respectively, were not significantly different from zero. The changes in blood flow to the mucosa occurred independent of cardiac output which remained stable during hyperventilation (Table 6.1).

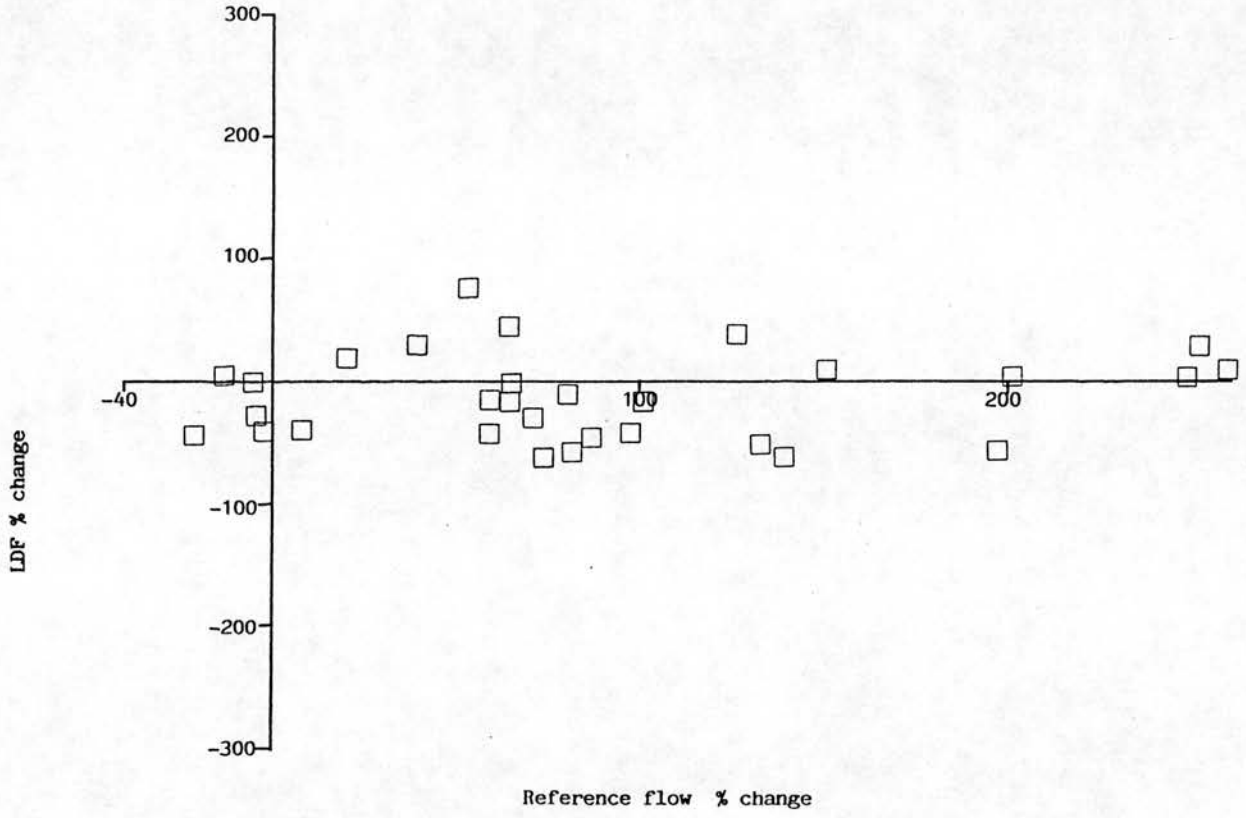


Figure 6.6 Changes in blood flow in each region of each dog measured by LDF and the reference flow technique during hyperventilation. Results are expressed as % change from baseline.

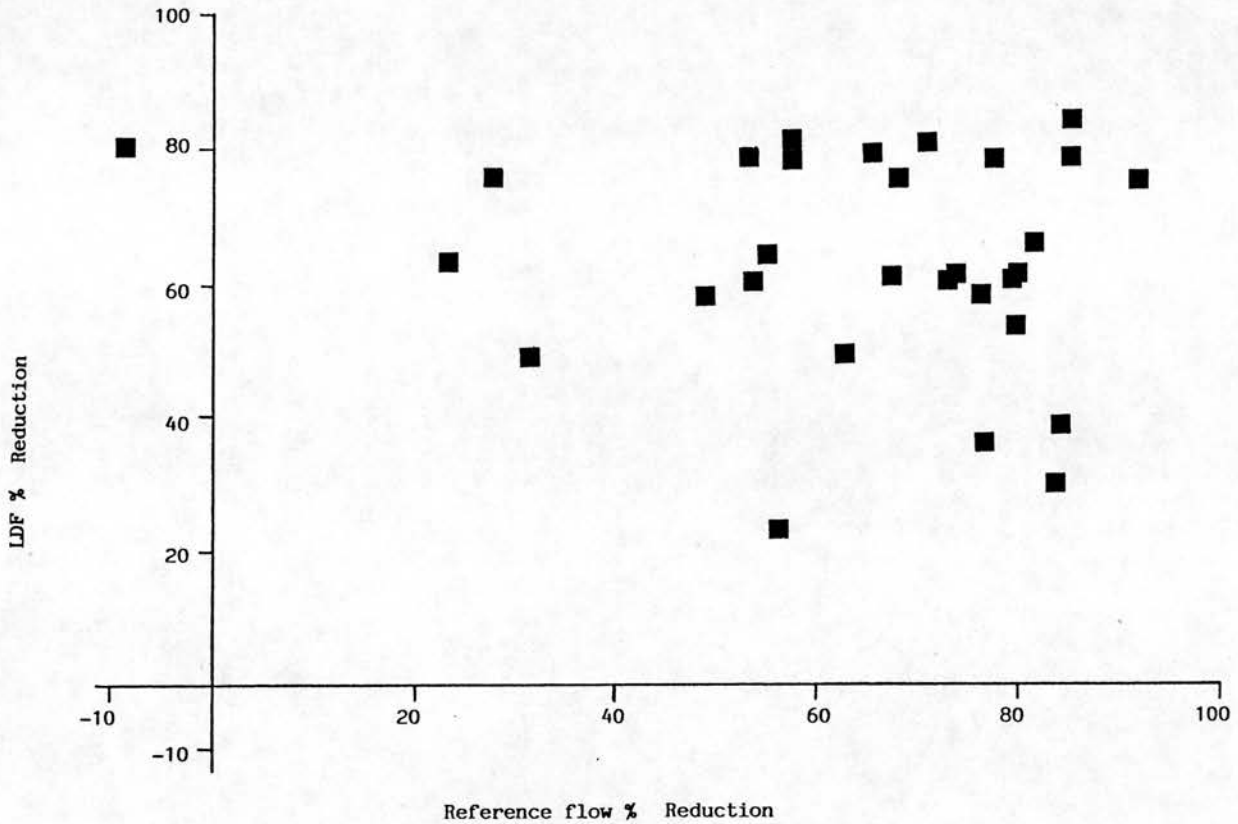


Figure 6.7. Changes in blood flow in each region of each dog measured by LDF and the reference flow technique during application of PEEP. Results are expressed as % reduction from baseline. Although both techniques indicate a fall in blood flow in all regions except one, there is no correlation between the techniques (r^2 = 0.02, p = NS).

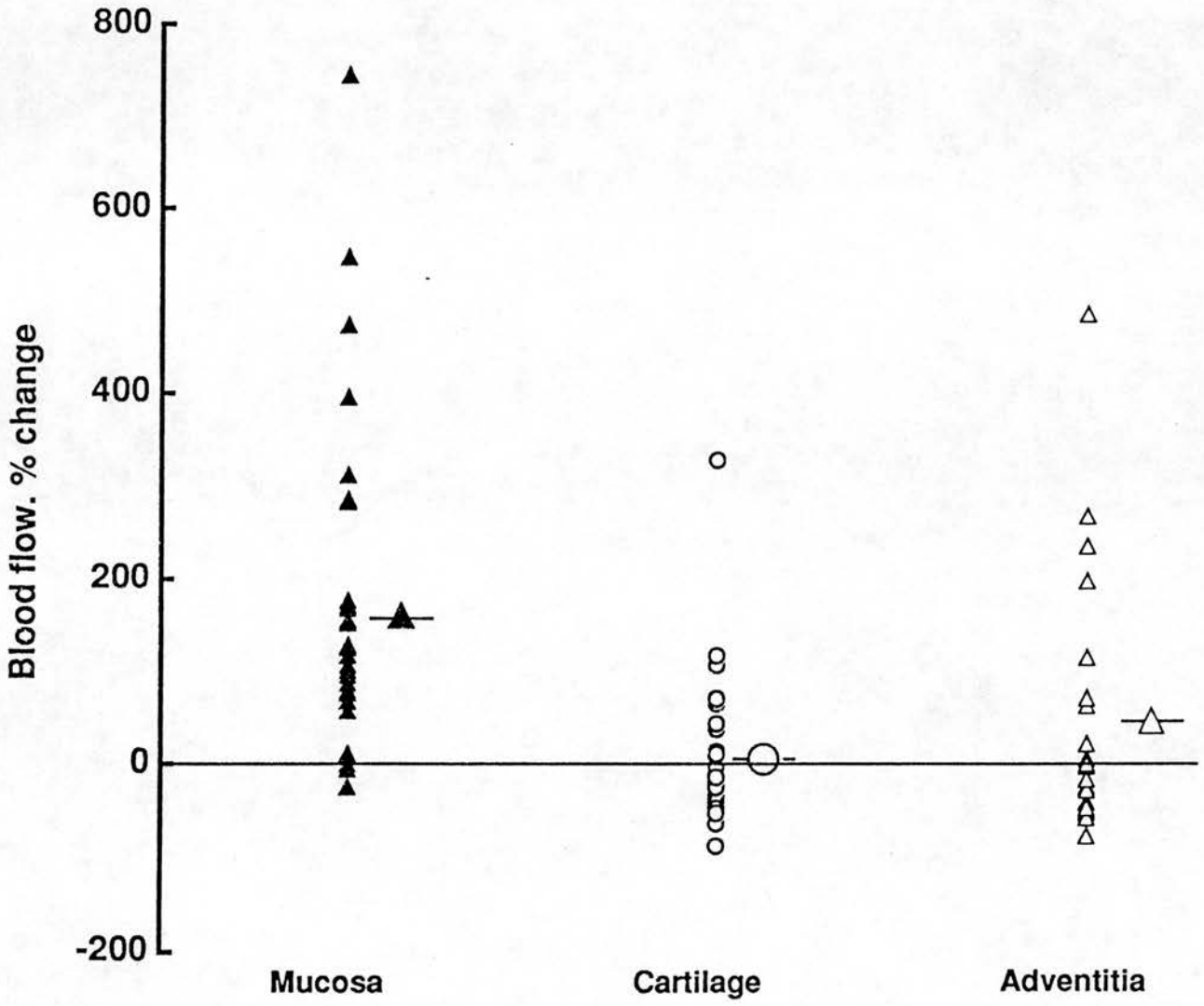


Figure 6.8. Changes in blood flow occurring during hyperventilation in each level of the airway wall are shown. Results are expressed as % change from baseline, and individual regional values and mean values are shown. The mean change in cartilage and adventitia is not significantly different from zero.

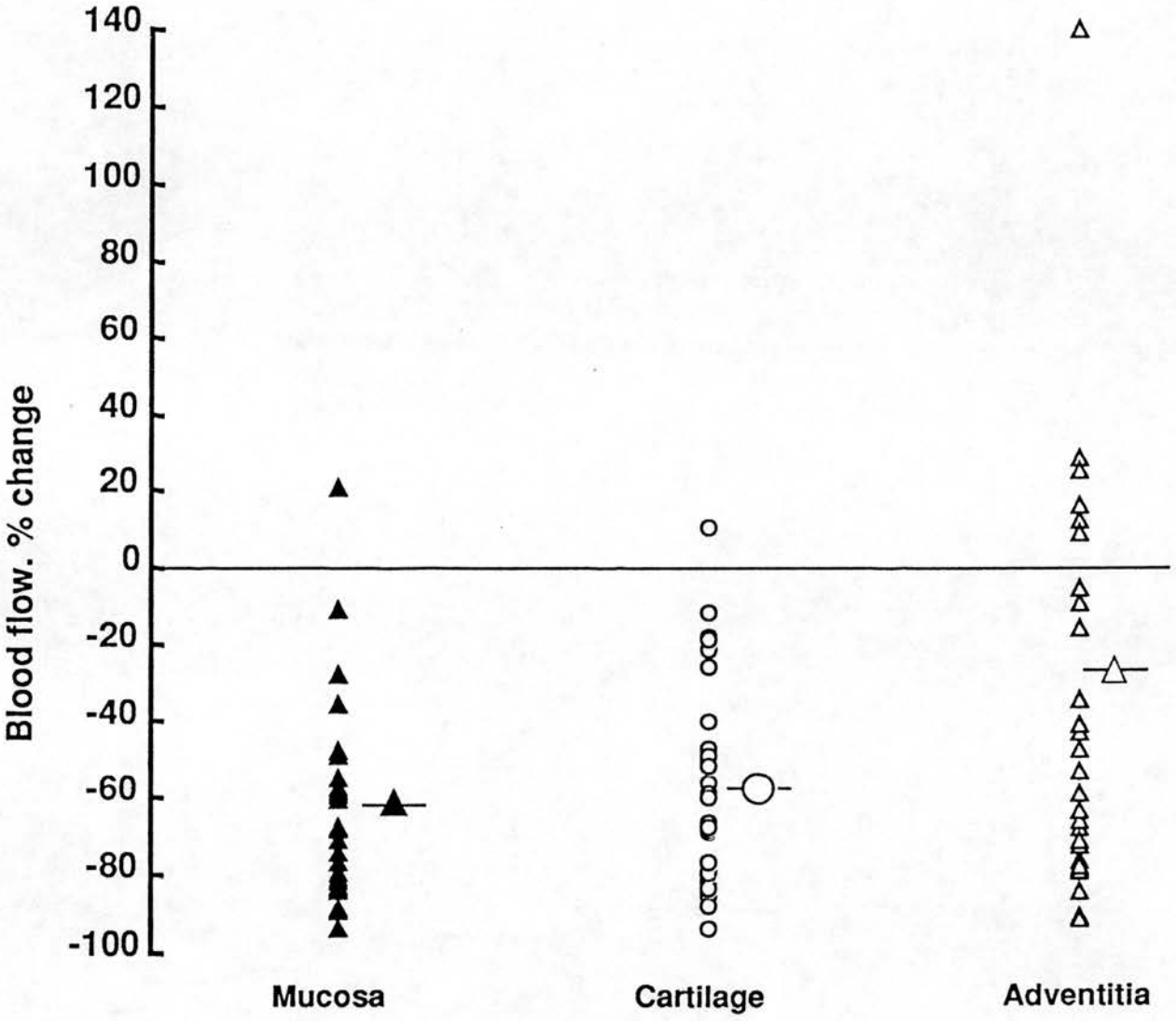


Figure 6.9. Changes in blood flow occurring during application of PEEP in each level of the airway wall are shown. Results are expressed as % change from baseline, and individual regional values and mean values are shown.

In contrast, responses to PEEP were much more uniform, a decrease in blood flow occurring in all three levels of the airway wall in most regions (figure 6.9). The magnitude of decrease was greatest in the mucosa (mean decrease 64 $\pm$ 25 %), less in the cartilage (58 $\pm$ 27 %), and least in the adventitia (35 $\pm$ 51 %). The fall in mucosal blood flow during PEEP was disproportionate to the fall in cardiac output which averaged 47 $\pm$ 17%.

6.5 DISCUSSION

The results of this study indicate that LDF measurements of blood flow in the tracheal wall of dogs differ substantially from measurements obtained using the radiolabelled microsphere reference flow technique, although some similarities are seen. During steady-state ventilatory conditions, a weak correlation does exist between the techniques, and this is highest during the application of PEEP. Site-to-site variation occurs in the LDF measurements within all regions of the trachea. Variation between regions in blood flow during baseline ventilation is of similar magnitude when measured by both techniques. During application of PEEP, both techniques demonstrate a decrease in tracheal blood flow from baseline values, and the mean decrease measured is similar with both techniques. However, when changes in blood flow in individual regions are examined, there is no correlation between the techniques. During hyperventilation of dry air, the reference flow technique demonstrates a substantial increase in tracheal blood flow, whereas the LDF measurements show variations in response, and no overall change in blood flow. However, the reference flow measurements also indicate that the change in blood flow during hyperventilation is not uniform throughout the tracheal wall. This latter observation must however be

interpreted with caution as will be discussed later.

The site-to-site and region-to-region variation in blood flow signals is similar to that seen in sheep and humans, and may reflect the anatomy of the microvascular bed in the airway wall. This will be further discussed in relation to other species in chapter 8.

The reason for the differences between the techniques in measuring responses to hyperventilation of dry air and the application of PEEP is not clear. However, there are several possible explanations. Since the LDF values are being compared with reference flow values, it is important to first consider sources of error in the reference flow method. This topic has been reviewed by Austin et al (1989).

In general, there are two major sources of error to be considered. Stochastic errors refer to the fact that microsphere entrapment and radioactive disintegrations occur at random rates and that the shape of the distributions of these events is related to the magnitude of the mean value. These events have been desribed by a Poisson distribution (Buckberg et al, 1971, Von Schweidler, 1905). Since the standard deviation of a Poisson distribution is the square root of its mean, the relative error (re) is as follows:

$$re = \frac{\sqrt{n}}{n} = \frac{1}{\sqrt{n}}$$

where n is the mean number of microspheres per sample. Thus to provide a relative error of 5%, it would be desirable to have at least 400 microspheres per tissue sample. From a knowledge of the number of spheres injected and the cardiac output, the number of spheres in any tissue sample can be calculated as shown in Appendix I. Applying this criterion to the tissue samples in this study, it is apparent that all of the mucosal samples have adequate spheres for accuracy $\pm$ 5%, but that the error in many of the cartilaginous and adventitial

samples may be higher due to lesser numbers of spheres in the tissue samples. The greatest relative error observed in any individual tissue sample was 61%, although in general the errors were of much smaller magnitude. The mean error for all tissue samples was 13.8 $\pm$ 16.7 % during baseline ventilatory conditions. A complete table of the error estimates for every tissue sample is shown in Appendix II. The conclusion regarding distribution of changes in blood flow must be interpreted with this potential error in mind. However, even if all tissue samples were subject to the maximum relative error, it would not alter the conclusion that changes in blood flow during hyperventilation are of much greater magnitude in the mucosa than in the underlying cartilage and adventitial layers. In the direct comparison of LDF measurements and total wall reference flow measurements on which figures 6.4 and 6.5 are based, all the tissue samples contain sufficient microspheres for acccuracy $\pm$ 5%.

The second source of error is methodological error. This includes errors due to circulatory impairment, flow biasing, nonentrapment, non-uniform mixing, aggregation, diameter variability, and the presence of additives in the microsphere preparation. These will be discussed in turn in relation to the present study.

Circulatory impairment due to deposition of microspheres is generally a transient phenomenon, and is dependent on the number and size of spheres injected. In a preparation in which LDF and radiolabelled micropheres were used to measure spinal cord blood flow (Lindsberg et al, 1989), it was shown that deposition of microspheres did reduce blood flow. The reduction peaked at 15 seconds after initiaton of injection, and the flow returned to pre-injection levels, presumably by the opening of collateral vessels, by one minute post injection. These observations correspond with vascular reactions to

microsphere injection studied by cinephotomicrography (Wiedeman and Tuma, 1977). However, in the present study, the time course of obtaining the LDF measurements was such that a temporary alteration in blood flow of this nature would have recovered before the second set of LDF measurements were obtained. When the LDF measurements before and after microsphere injection are compared, there is a wide scatter of responses, with LDF measurements being higher in some areas after microsphere injection. The mean difference in LDF flow signals within regions before and after microsphere injection in the present study is -0.53 LDF units with a standard deviation of 4.16, indicating that the change is not significantly different from zero. Thus it is unlikely that circulatory impairment due to microsphere deposition is an important factor in the present study.

Flow biasing is a another potential source of error. Microspheres of larger diameters have been shown to preferentially enter regions of higher flow (Domenech et al, 1969), and this has been a particular problem in fractionating flow within the gut wall (Maxwell et al, 1981). However, the microspheres used in this study are of very uniform size, the standard deviation quoted by the manufacturers being 0.1 microns. Microspheres of this size have been shown in gut wall blood flow measurements to distribute evenly between the muscularis and a lumped mucosal-submucosal tissue level (Maxwell et al, 1981), and uneven distribution related to flow would therefore seem unlikely to be a major source of error in the present study.

The possibility of non-entrapment due to microsphere arterio-venous shunting will be discussed later, but is unlikely to be a significant problem in the tracheal wall of dogs.

Strenuous attempts were made to ensure adequate mixing of mmicrospheres and to avoid aggregation of spheres during the injection

procedure. The spheres were suspended in 0.9% saline with a surfactant solution (0.01% Tween 80), and were dispersed in a sonicator for 2 minutes prior to injection. The site of injection into the left ventricle should allow for adequte mixing of spheres with blood.

Counting errors within the scintillation counter were generally of small magnitude. An example of actual counts determined during one experiment is shown in Appendix 3. The counting errors derived from the linear regression are shown and are generally less than 4%.

These observations, combined with results from previous studies using the microsphere reference flow method in this laboratory suggest that the technique is reliable and reproducible. In Baile's original report of the microsphere reference blood flow method (1982), reproducibility was examined by injecting microspheres with three separate radiolabels simultaneously in one group of dogs, and at thirty minute intervals in another group. Although there was variation in tracheal blood flow measurments between dogs, there was no significant difference between the three separate measurements of blood flow within each dog during either protocol. Airway blood flow responses to hyperventilation and PEEP have also been very consistent in previous studies in the same laboratory (Baile et al, 1984, 1987 a and b). Considered together, these results suggest that the above potential sources of error in the reference flow technique do not explain the lack of concordance with the LDF measurements.

The lack of concordance may be a result of the LDF technique, as used in this study, failing to accurately reflect microvascular flow, or detecting a different type or distribution of blood flow.

It is possible is that the LDF measurements are unreliable, due to excessive "noise" (i.e. flow signal derived from sources other than

blood flow) in relation to the blood flow signal obtained. In this study, the highest correlation between the LDF signals and the reference flow measurements from different regions was observed during the application of PEEP. Application of PEEP may reduce noise, both by reducing cardiac output and therefore transmitted cardiac pulsation, and by acting as a pneumatic splint in the airway, further reducing airway vibration due to transmitted pulsation. In the studies in sheep described in the previous chapter, "noise" was found to account for about 20% of the laser Doppler signal. However, the application of PEEP is also associated with lower absolute levels of blood flow, and it may be that the LDF instrument is more accurate at low flows. Shepherd et al (1987) measured gastric mucosal blood flow using the same model of laser Doppler flowmeter and demonstrated linear responses with the instrument up to total gastric blood flow values of 300ml/min/100g tissue. These observations suggest that the absolute levels of blood flow in our preparation are not an important factor in the lack of concordance.

Of more importance may be the characteristics of the two types of blood flow measurement, and the complex nature of the vascular bed studied. Measurements of flow made using laser Doppler flowmetry are tissue volume dependent, and the depth of measurement of the LDF probe in the airway wall is not known. The theory of laser Doppler flowmetry indicates that the depth of measurement is determined in part by the distance between the transmitting and receiving fibres, and in part by the type of laser used (Bonner and Nossal, 1981). With a fibre separation of 0.5 mm, as in this instrument, a measurement volume of about 1 mm^3 would be expected. There is marked variability in the density of the capillary bed in the tracheal wall (Laitinen et al,

1987d), and it has also been demonstrated that, in dogs, the thickness of the mucosa and submucosa in which the capillary plexus lies may vary from 150 to 900 microns (Laitinen et al, 1986b). This means that the proportions of submucosal and cartilaginous vascular bed sampled by the LDF probe may vary considerably from site to site. Furthermore, these proportions may vary over time at the same site if mucosal vascular engorgement or oedema occurs. The reference flow data in this study indicate that blood flow to the mucosa and submucosa, expressed per unit mass of tissue, is 25 times greater than blood flow to the cartilaginous portion under baseline conditions, and that the vascular response to hyperventilation is not uniform throughout the airway wall. Thus, it is possible that the LDF signal is derived from different proportions of mucosa and underlying wall before and after hyperventilation, resulting in the discrepancy in responses between regions. Furthermore, since some of the larger arterioles in the airway may lie within 1 mm of the mucosal surface, detection of flow in these vessels might account for high LDF signals at some sites, whilst flow in these vessels would not be reflected by the reference flow technique. During application of PEEP, on the other hand, the change in blood flow throughout the airway wall is more uniform, and the changes in blood flow detected by both techniques are qualitatively similar throughout the regions, though still not quantitatively similar.

Another potential source of discrepancy between the techniques also relates to the anatomy of the tracheal wall vasculature. The reference flow technique relies on trapping of microspheres in capillaries which are less than 15 microns in diameter. It is therefore theoretically possible that blood may flow from the arterial to the venous systems through vessels of larger internal diameters,

constituting a shunt. Such flow would be detected by the laser Doppler system, but not by the reference flow technique. Studies in rabbits (Hughes, 1965), and in sheep (Hill et al, 1989) have demonstrated the presence of venous sinuses in the airway wall, which are of large diameter, and through which flow would not be detected by 15 micron diameter microspheres. There is however no anatomical evidence for such vessels in dogs. That shunting of this type does not occur in the canine airway is also supported by previous data from Baile et al (1987b). In that study, both 15 and 50 micron diameter microspheres were injected simultaneously to measure the blood flow response to hyperventilation of warm and dry air in dogs. No difference in the airway blood flow response was seen whether 15 or 50 micron diameter spheres were used, although in 2 dogs, parenchymal blood flow appeared 3 to 4 times greater when measured by the 50 micron spheres than when 15 micron spheres were used. These results suggest that there are arteriovenous anastomoses in the parenchyma of canine lungs of between 15 and 50 microns size, but no such anastomoses in the airways.

It is possible that blood flow to the airway wall derived from a source other than the systemic vessels occurred, and was detected by the LDF system. Since the radioactive microspheres were injected into the left ventricle, and assuming that large vascular channels of the type described above are not present in canine airways, then all systemic blood flow to the airways should be measured by the reference flow technique. However, blood flow derived from the pulmonary circulation would not be reliably detected. Barman et al (1988) have recently demonstrated that, in dogs, up to 50% of the blood flow to the bronchi may be derived from the pulmonary circulation, but only a small proportion of the tracheal blood flow (about 3%) is derived from

this source, and this is unlikely therefore to be of major significance in the present study.

Another likely source of discrepancy between the LDF and reference flow values is that due to temporal and spatial variations in microvascular anatomy and physiology, the sampling method used for the LDF values in this study did not provide a representative value for the tissue region. It is not possible, having moved the LDF probe away from a particular site, to subsequently return the probe to the identical site. The major difference between this study and that described in the previous chapter, is that in the previous study the probe was held at each individual site until the entire protocol of flow changes was complete. In this study, the probe was moved between sites between interventions designed to alter blood flow. Whilst it was hoped that averaging LDF values from several sites would provide a flow signal representative of each region, it appears likely that this sampling was inadequate. This problem will be further discussed in chapter 8.

In summary, the results of this study suggest that although there is some concordance between LDF and reference flow measurements, the LDF technique, when used in this fashion, does not accurately reflect tracheal wall blood flow in the dog, especially at higher levels of flow such as those occurring during hyperventilation of cold air. The most likely reasons for this discrepancy are the detection of "noise" by the LDF system, the non-uniformity of blood flow changes in the tracheal wall, and the sampling of an inadequate number of sites by the LDF technique to reflect overall perfusion of a tissue region.

CHAPTER 7

MEASUREMENT OF AIRWAY WALL BLOOD FLOW BY LASER DOPPLER FLOWMETRY IN HUMANS

7.1 SUMMARY

Measurements of airway wall blood flow were obtained using laser Doppler flowmetry during bronchoscopy in human volunteers. Eight subjects who were undergoing cardiac surgery were studied during cardiopulmonary bypass, and nine subjects were studied during bronchoscopy while they were awake and breathing spontaneously. Site-to-site variation in LDF signals was substantial in both groups, the mean (SD) coefficient of variation (CV) being 19.5 (13.6)% in the cardiopulmonary bypass patients, and 23.2 (13.8)% in the spontaneously breathing subjects. The CV between regions of the airways in the cardiopulmonary bypass group was 51 (12)%. LDF measurements during hyperventilation of cold, dry air and warm, humid air in non-asthmatic subjects showed no definite trend. The results indicate that LDF measurements can be obtained in the airways of awake humans, and they share the characteristics of measurements in other species. However, further investigation and development of the technique will be necessary before the results obtained can be meaningfully interpreted.

7.2 INTRODUCTION

This study had three main objectives: to examine the feasibility of obtaining laser Doppler flow measurements in the airways of awake human subjects, to assess site-to-site variation in LDF measurements in the airways in order to allow comparison with the other species already described, and to examine the influence of respiratory and cardiac artifacts on LDF measurements. The latter objective was undertaken by studying both awake, spontaneously breathing volunteers, and patients undergoing cardiopulmonary bypass for coronary artery surgery. In addition, the influence of isocapnic hyperventilation of cold or dry air on LDF measurements in the trachea was examined in a small group of normal and asthmatic subjects.

7.3 SUBJECTS AND METHODS

Eight patients undergoing cardiopulmonary bypass for coronary artery surgery were studied during anaesthesia, and nine volunteers were recruited for studies performed during bronchoscopy while the subject was awake and breathing spontaneously. All subjects gave written informed consent to participate, and the studies were approved by the Ethical Committee of St Pauls Hospital, Vancouver, where the studies were carried out.

7.3.1 Studies during cardiopulmonary bypass

Eight subjects, mean age 63 $\pm$ 8 years were studied during anaesthesia. Two were current smokers, 5 were ex-smokers, and 1 was a non-smoker. All were anaesthetised and intubated, undergoing coronary artery bypass grafting carried out using standard techniques. Measurements of airway wall blood flow were made during cardiopulmonary bypass. At this stage of the surgery, the patient is

fully heparinized, ventilation is temporarily arrested, and 5 cm H_2O PEEP is applied to maintain some degree of lung inflation. Cannulae are inserted into the aorta, and into the superior and inferior vena cava, a sump is placed into the apex of the left ventricle via the right superior pulmonary vein, and caval tapes are applied. The patient is cooled systemically (28°C), the aorta is cross-clamped, and the heart is arrested and cooled with 1000ml of cold potassium cardioplegic solution. Blood is then withdrawn from the caval cannulae, passed through an oxygenator and heat exchanger, and returned to the aorta by means of a roller pump. Since the pulmonary circulation is not perfused other than by systemic blood passing through the bronchopulmonary anastomoses, the only source of blood supply to the airway wall is via the bronchial circulation. Since both respiration and cardiac pulsation are arrested, motion artifacts in the LDF signal related to these sources do not occur.

In each subject, an Olympus IT10 bronchoscope was introduced into the trachea at this stage of the surgery, and the LDF probe was inserted through the suction channel. Measurements of airway wall blood flow were made as described in chapter 3. The tip of the LDF probe was advanced to 2cm distal to the tip of the bronchoscope and allowed to rest on the airway mucosa. Measurements were obtained in each subject at 4 sites in each of 4 regions, namely the posterior wall and the right lateral wall of the trachea about 1 cm proximal to the main carina, and the posterior walls of the right and left main bronchi, 1-2 cm distal to the main carina. The probe was allowed to rest in position for about 5 seconds at each site. An averaging time of 1 second was used for the LDF signals. The LDF value for each site was taken as the mean value determined by eye from the flow tracing.

7.3.2. Studies in Spontaneously Breathing Awake Subjects

Nine volunteers were recruited for these studies. Anthropomorphic data for these subjects are shown in table 7.1. All were male and their mean age was 33 ± 8 years. Four gave a history of recurrent wheeze requiring therapy and had a diagnosis of asthma established by their physicians, and the other five had no history of current or previous respiratory disease.

Each of the non-ashmatics subjects was studied on three days. On one day, the effect of a period of eucapnic hyperventilation of cold, dry air on airway calibre as measured by forced expiratory volume in one second (FEV1) was studied. On a second day, airway blood flow as measured by LDF during bronchoscopy was studied during an identical challenge. On a third day, airway blood flow as measured by LDF during bronchoscopy was again studied during eucapnic hyperventilation of warm, humid air. In one asthmatic subject, who had airway calibre and airway blood flow responses to cold air hyperventilation measured while he was receiving treatment with inhaled beclomethasone and salbutamol, no change in airway calibre occurred during or after hyperventilation. For the purposes of analysis, the data from this subject were therefore included with the non-asthmatic subjects. In the three asthmatic subjects who did develop bronchoconstriction associated with cold air hyperventilation, the effects of hyperventilation of cold air on airway blood flow measured by LDF during bronchoscopy were similarly studied on a separate occasion. A humid air challenge was not performed in this group.

TABLE 7.1 HUMAN VOLUNTEERS – ANTHROPOMORPHIC DATA

SUBJECT	AGE (yr)	HEIGHT (in)	ASTHMATIC Y/N	FEV1 (%PRED)	FVC (%PRED)	FEV/VC %
PP	43	73	N	105	92	91
RG	37	69	N	104	99	85
GM	28	75	N	86	95	73
JM	19	68	N	105	108	86
BW	46	70	Y	105	127	63
CH	30	68	N	96	89	89
JL	32	67	Y	76	95	66
MK	34	67	Y	95	100	78
GO	29	67	Y	84	95	75

7.3.3 Cold air generation

Cold air was generated for inhalation by passing the inspired room air through a heat exchanger, comprised of a tube containing an insulated coil system through which ethanol, which had been cooled to -35°C in a refrigerated chamber, was pumped. A copper constantin thermocouple (0.005in diameter, 95% response time ~0.5 sec in air) was mounted in the inspiratory limb of the breathing circuit to measure inspired temperature. The subject breathed through a 2-way valve, the expiratory limb of which contained a turbine spirometer to measure expired minute volume, and an infrared CO_2 analyser (Capnograph, Hewlett Packard) to measure end-tidal CO_2. Carbon dioxide was added during hyperventilation as necessary to the inspired limb of the circuit to maintain end- tidal CO_2 at 35-40 mm Hg (figure 7.1).

7.3.4 Warm, humid air generation

Warm, humid air was generated for inhalation by humidifying room air using an ultrasonic nebuliser (Devilbiss 65, setting 1-2), and passing it through a series of copper coils in a water bath heated to approximately 50°C. Inspired temperature was measured as before using a thermocouple mounted in the inspired limb of the circuit. End-tidal CO_2 was measured as before and CO_2 was added as necessary to inspired air to maintain eucapnic conditons during hyperventilation (figure 7.2).

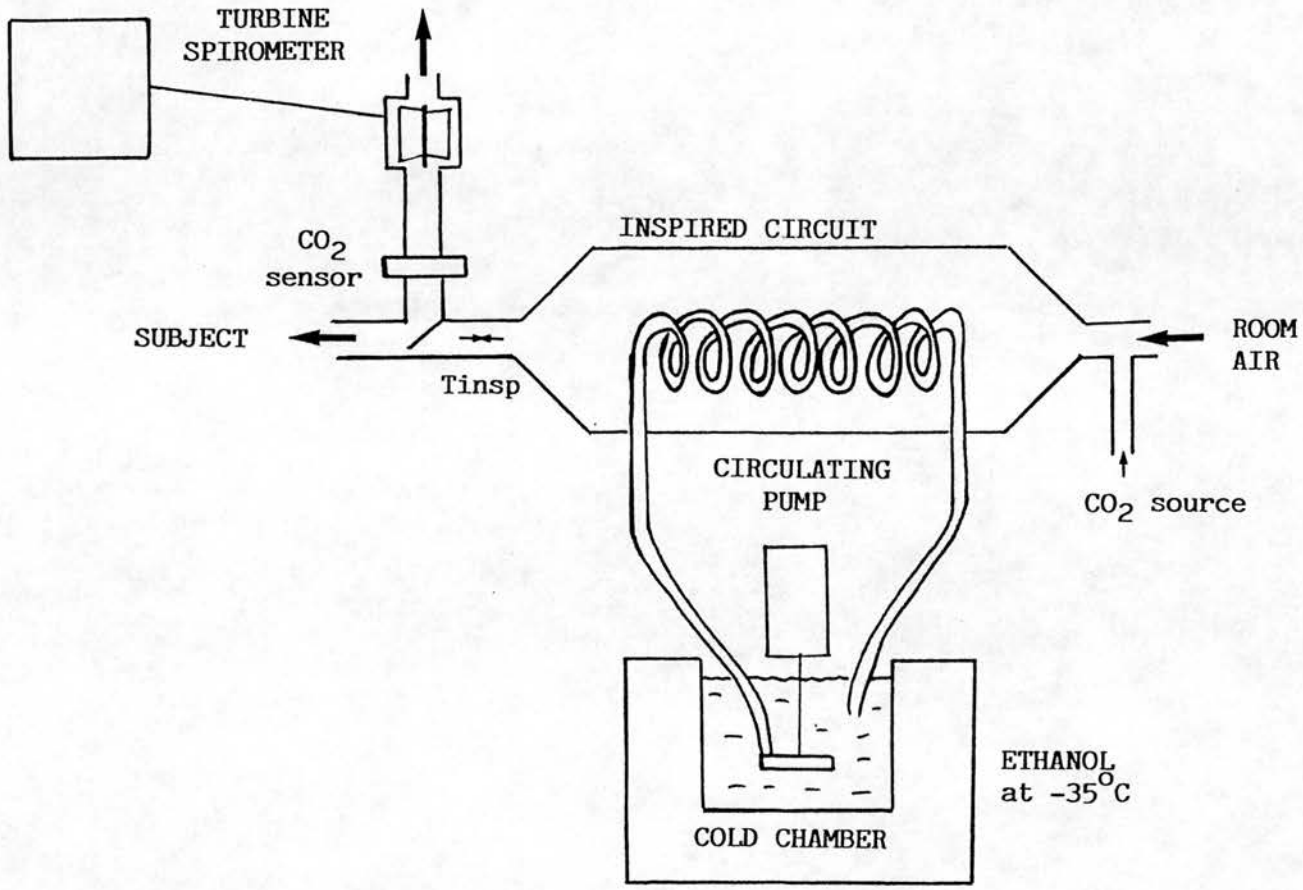


Figure 7.1. Schematic diagram of apparatus for cold air generation.

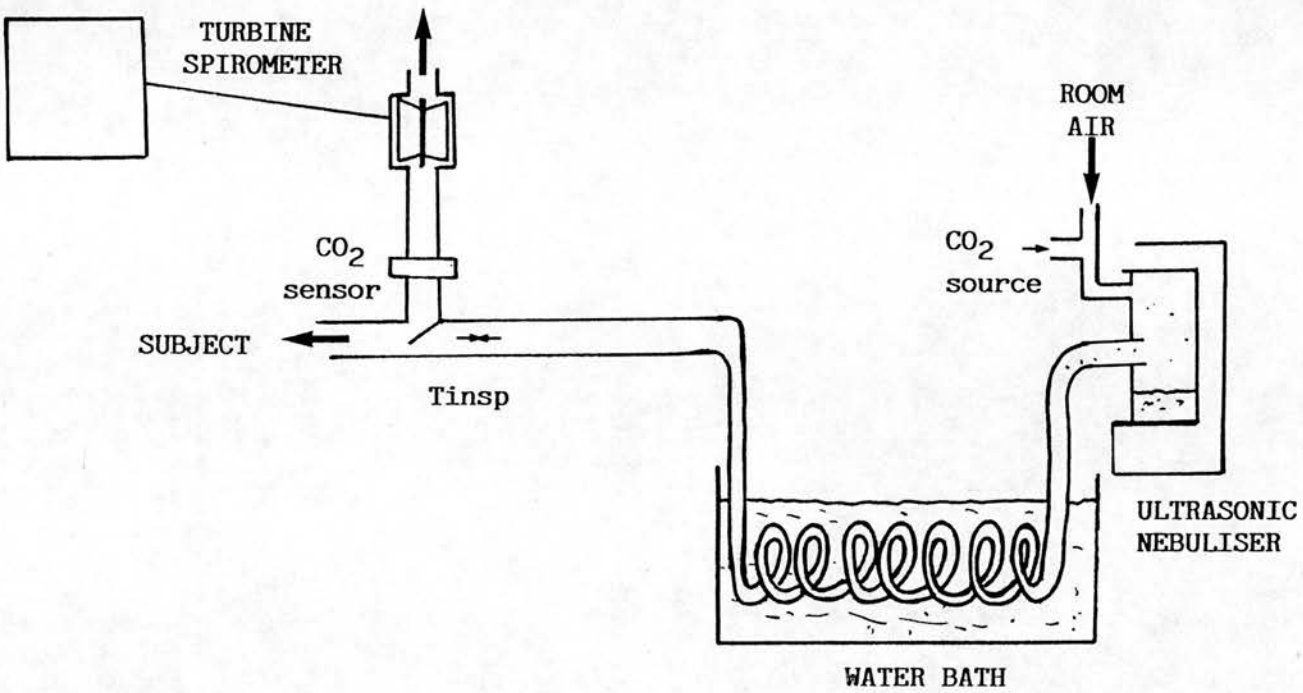


Figure 7.2. Schematic diagram of apparatus for humid air generation.

7.3.5 Protocol for airway calibre measurements

Forced expiratory volume in one second (FEV1) and forced vital capacity (FVC), best of three attempts, were measured using a dry wedge spirometer. Subjects were then asked to hyperventilate cold air using the apparatus described above for a period of 8 minutes. Single measurements of FEV1 were repeated at 2 minute intervals during the period of hyperventilation, and at one minute intervals during a recovery period of 8 minutes during which the subjects were tidally breathing room air. Inspired temperature and minute ventilation were recorded at one minute intervals during hyperventilation. End tidal CO_2 was continuously monitored during hyperventilation, and CO_2 was added as necessary to the inspired air to maintain eucapnic conditions. Hyperventilation was discontinued before completion of the 8 minute period if the subject developed significant bronchoconstriction, defined as a fall in FEV1 of greater than 15% from the baseline value. Table 7.2 summarises the ventilation achieved and the inspired temperatures measured. The efficacy of the apparatus in generating cold air was variable, resulting in differing levels of inspired temperature between subjects.

TABLE 7.2 INSPIRED TEMPERATURE AND VENTILATION DURING COLD AIR CHALLENGE AND SPIROMETRY

SUBJECT	Ti (°C)	V_e (Litres)
1	-4	77
2	4	76
3	-12	71
4	-11	50
5	-18	59
6	0	76
7	3	49
8	2	74
9	37*	40

* This subject underwent challenge with warm, dry (cylinder) air.
Ti, inspired temperature, V_e, expired volume.

7.3.6 Protocol for bronchoscopy and LDF measurement

Subjects were fasted for four hours prior to bronchoscopy. They were then seated in a semi-recumbent position on an examination couch, and an intravenous cannula was inserted into a forearm vein. Premedication with atropine, 0.6mg IV, was given 10 minutes before bronchoscopy to reduce airway secretions and protect against vasovagal syncope during insertion of the bronchoscope. The electrocardiogram was continuously monitored during bronchoscopy, as was arterial oxygen saturation using a transcutaneous pulse oximeter.

The anterior nares were anaesthetised using topical cocaine (1 - 3 ml of 5% solution) and the nasopharynx, larynx and vocal cords were anaesthetised using lignocaine (7 - 11 ml of 2% solution). The fibreoptic bronchoscope was then inserted transnasally, and a further 2-3 ml of lignocaine solution was injected into the trachea through the suction channel of the bronchoscope. LDF measurements were obtained as in the previous animal studies. The tip of the LDF probe was extended 2-3 cm distal to the tip of the bronchoscope and was allowed to rest on the mucosa in the mid portion of the trachea, 4 cm from the main carina, under direct vision. Each LDF measurement was obtained during a brief period of breath holding at end expiration. Fifteen baseline measurements of tracheal blood flow were made during quiet tidal breathing, and subjects were then asked to hyperventilate cold, dry or warm humid air for a period of 8 minutes (hyperventilation period), and thereafter to return to quiet tidal breathing for a further 8 minutes (recovery period). Tracheal blood flow measurements were made in triplicate at two minute intervals during the period of hyperventilation and at one minute intervals during the recovery period. For analysis, the blood flow data were averaged over the first and second halves of the hyperventilation

period (HV1 and HV2), and over the first and second halves of the recovery period (REC1 and REC2). Tables 7.3 and 7.4 summarise the ventilation achieved and the inspired temperatures during bronchoscopy.

TABLE 7.3 INSPIRED TEMPERATURE AND VENTILATION DURING COLD AIR CHALLENGE AND BRONCHOSCOPY

SUBJECT	Ti (°C)	V_e (Litres)
1	−12	92
2	−12	93
3	+10	73
4	−49	55
5	−29	59
6	−10	66
7	+15	27
8	−3	70
9	−20	43

Ti, inspired temperature, V_e, expired minute volume.

TABLE 7.4 INSPIRED TEMPERATURE AND VENTILATION DURING HUMID AIR CHALLENGE AND BRONCHOSCOPY

SUBJECT	Ti (°C)	V_e (Litres)
1	32	64
2	33	87
3	38	70
4	35	57
5	40	66
6	42	62

Only non-asthmatic subjects (1-6) underwent humid air challenge.
Ti, inspired temperature, V_e, expired minute volume.

7.4 DATA ANALYSIS

For the anaesthetised subjects undergoing cardiopulmonary bypass, the mean value and the coefficient of variation (CV) was calculated for the four sites within each region and the four regions within each subject, and the CV between subjects was also calculated. For the spontaneously breathing awake subjects, the mean value and the CV for the fifteen measurements obtained during baseline conditions was calculated. LDF values during baseline, hyperventilation and recovery periods were compared by single factor analysis of variance (Campbell, 1974).

7.5 RESULTS

No complications occurred associated with the LDF measurements in either group, and no additional discomfort was experienced in the awake subjects other than that associated with bronchoscopy.

Specimen tracings from an anaesthetised subject during cardiopulmonary bypass and an awake subject during breath-holding are shown in figure 7.3. The flow pattern in the awake subject shows large fluctuations related to cardiac pulsation, while that in the cardiopulmonary bypass patient shows minor fluctuations only related to the flow pattern imposed by the perfusion pump.

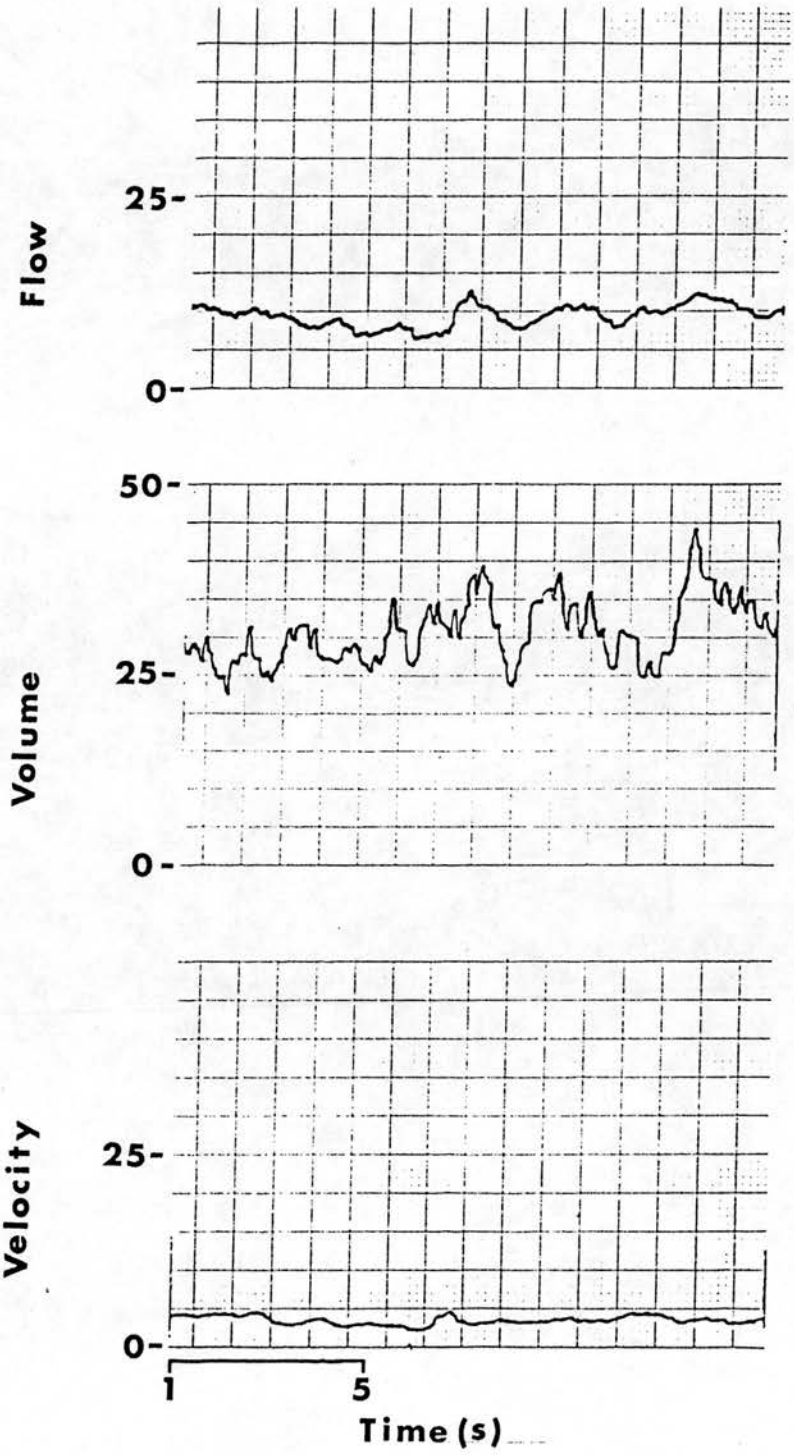


Figure 7.3 (a). Specimen tracing obtained by LDF in the mid portion of the trachea of a subject during cardiopulmonary bypass. Minor fluctuations only occur in each tracing (cf Figure 7.3 (b)).

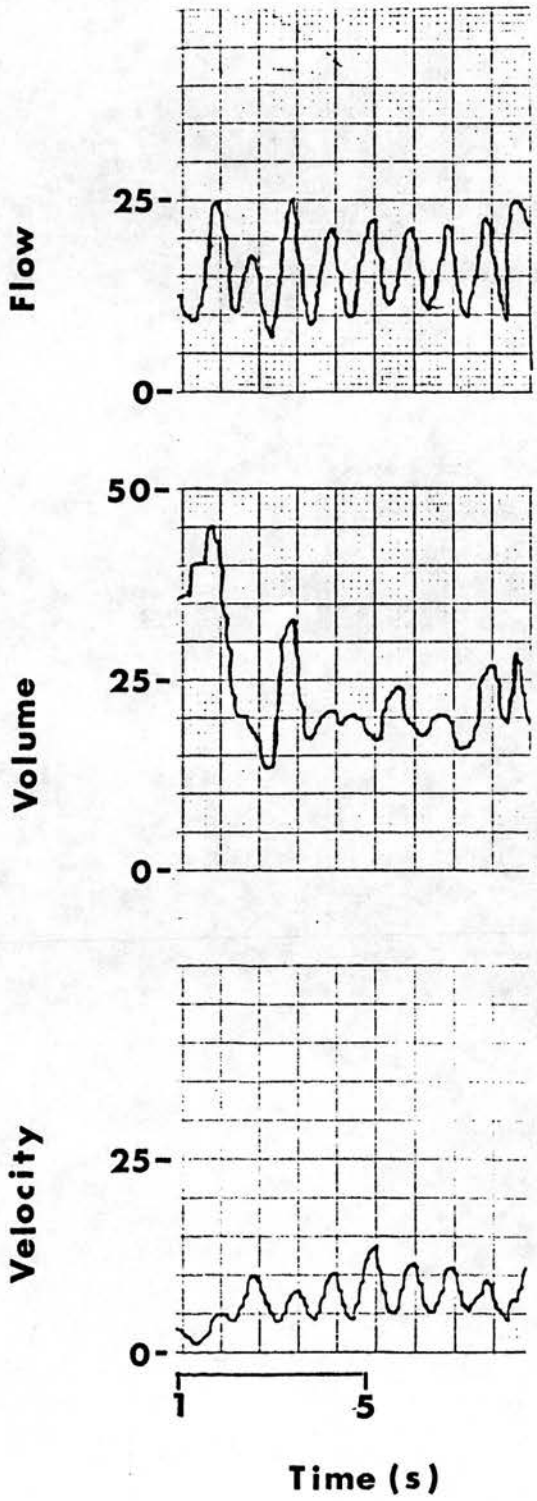


Figure 7.3 (b). Specimen tracing obtained by LDF in the mid portion of the trachea of an awake subject during breath-holding. Oscillations due to cardiac pulsation are prominent.

The absolute LDF flow signals observed and the coefficients of variation (CV) between sites for measurements made on the lateral wall of the trachea are shown in tables 7.5 and 7.6. There was wide intersubject variation in LDF flow measurements in both groups, but in general, the absolute magnitude of the LDF signals was similar in the cardiopulmonary bypass group and in the awake, non-asthmatic volunteers. Coefficients of variation between sites also varied widely between subjects, but was similar on average in the cardiopulmonary bypass group and in the spontaneously breathing subjects. For the cardiopulmonary bypass patients, the CV between regions was 51 ($\pm$12) %.

The airway calibre responses to hyperventilation of cold, dry air in the asthmatic and non-asthmatic subjects are shown in table 7.7 and in figures 7.4 and 7.5. The asthmatic subjects (numbers 7 to 9) developed a significant fall in FEV1 during the recovery period after breathing cold air, the maximum fall observed exceeding 15% from baseline in each of the subjects. The non-asthmatic subjects (subjects 1 to 5) had only minor changes in FEV1, with the exception of subject 1, in whom an 18% fall from baseline occurred 6 minutes after stopping hyperventilation.

The LDF responses to hyperventilation of humid air and cold air in the non-asthmatic subjects are shown in tables 7.8 and 7.9 and in figures 7.6 and 7.7. LDF responses to cold air hyperventilation in the asthmatic subjects are shown in table 7.10 and figure 7.8. There was a tendency for LDF signals to increase during hyperventilation of warm, humid air, though this did not achieve statistical significance. During cold, dry air hyperventilation, there was great variation in response in both the normal and the asthmatic subjects, and no overall trend was apparent in this small group of subjects.

TABLE 7.5 LDF TRACHEAL FLOW MEASUREMENTS IN SUBJECTS UNDERGOING CARDIOPULMONARY BYPASS

SUBJECT	MEAN	SD	CV(%)
1	25	2.7	10.9
2	31.4	7.4	23
3	29.5	3.5	7.9
4	3.5	0.6	15.9
5	6.4	0.8	11.7
6	13.5	5.8	43
7	8.8	3.2	36.7
8	12.4	0.9	6.9
mean	16.3		19.5
SD	10.8		13.6

Values are in LD units. SD, standard deviation, CV, coefficient of variation.

TABLE 7.6 LDF TRACHEAL FLOW MEASUREMENTS IN AWAKE SUBJECTS

SUBJECT	A/NA	MEAN	SD	CV(%)
1	NA	3.7	1.8	48.6
2	NA	7.3	0.7	9.5
3	NA	34.8	6.6	18.9
4	NA	18.3	6.6	27.7
5	NA	6.4	1.0	15.6
6	NA	26.5	5.0	18.8
mean		16.1		23.2
SD		12.5		13.8
7	A	36.4	4.0	10.9
8	A	15.1	6.8	45
9	A	21.8	7.9	36.2
mean		24.4		30.6
SD		10.9		17.7

Values are in LD units. A, asthmatic, NA, non-asthmatic, SD, standard deviation, CV, coefficient of variation.

TABLE 7.7 FEV1 DURING HYPERVENTILATION OF COLD AIR IN ASTHMATIC AND NON-ASTHMATIC SUBJECTS

SUBJECT	1	2	3	4	5	6	7	8	9
	NA	NA	NA	NA	A/NA	NA	A	A	A
BASELINE	4.1	4	3.91	3.97	3.6	3.83	2.7	3.36	3.13
MIN 2	3.74	4.1	3.84	3.93	3.54	3.88	2.41	3.3	3.17
MIN 4	3.8	4.1	3.62	3.83	3.5	3.83	2.05	3.13	3.22
MIN 6	3.79	3.97	3.63	3.83	3.59	3.79		3.22	3.03
MIN 8	3.79	4.1	3.82	3.89	3.62	3.83		3.18	3.18
MIN 9	3.55	3.8	3.76	3.79	3.44	3.7	1.3	3.04	2.85
MIN 10	3.55	3.93	3.7	3.88	3.49	3.7	1.6	3.18	2.7
MIN 11	3.4	3.97	3.64	3.83	3.47	3.74	1.4	3.13	2.48
MIN 12	3.5	3.93	3.81	3.7	3.42	3.74	1.6	2.85	2.8
MIN 13	3.55	3.93	3.81	3.74	3.54	3.74	1.66	2.99	3
MIN 14	3.36	3.93	3.74	3.74	3.6	3.83	1.6	2.85	2.99
MIN 15	3.41	3.93	3.85	3.83	3.53	3.74	1.4	2.99	2.94
MIN 16	3.6	3.93	3.82	3.83	3.54	3.83	1.5	2.95	3.04

FEV1 values are in litres. NA, non-asthmatic, A, asthmatic. A/NA is an asthmatic subject whose data was included with the non- asthmatic group for analysis. Hyperventilation was performed from baseline to minute 8, followed by an 8-minute recovery period. Subject 7 developed marked bronchoconstriction after 4 minutes and hyperventilation was stopped at that point.

TABLE 7.8 LDF MEASUREMENTS DURING HYPERVENTILATION OF HUMID AIR IN NON-ASTHMATIC SUBJECTS

PERIOD SUBJECT	CONTROL	HV1	HV2	REC1	REC2
1	3.73	6.33	9.00	6.25	5.57
2	7.33	9.17	19.67	21.75	10.73
3	34.75	21.33	17.83	22.17	35.67
4	18.28	26.83	19.33	24.58	
5	6.4	10.5	11.67	14.58	8.42
6	26.53	24.83	37.83	36.55	36.18
mean	16.17	16.50	19.22	20.98	19.31
SD	12.53	8.86	10.1	10.15	15.27

Measurements are in LDF units. HV1, first four minutes of hyperventilation, HV2, second four minutes of hyperventilation. REC1, first four minutes of recovery, REC2, second four minutes of recovery.

TABLE 7.9 LDF MEASUREMENTS DURING HYPERVENTILATION OF COLD AIR IN NON-ASTHMATIC SUBJECTS

PERIOD SUBJECT	CONTROL	HV1	HV2	REC1	REC2
1	8.8	7.17	8.67	8.17	8.83
2	18.7	14.33	13.8	16.89	22.92
3	27.2	17	13.7	17.8	14.3
4	12.27	12.17	27.33	17.3	
5	10.8	8.83	10.16	9.92	4.08
6	9.07	25.33	18.33	23	16.71
mean	14.47	14.14	15.33	15.51	13.37
SD	7.21	6.54	6.77	5.51	7.25

Measurements are in LDF units. HV1, first four minutes of hyperventilation, HV2, second four minutes of hyperventilation, REC1, first four minutes of recovery, REC2, second four minutes of recovery.

TABLE 7.10 LDF MEASUREMENTS DURING HYPERVENTILATION OF COLD AIR IN ASTHMATIC SUBJECTS

PERIOD SUBJECT	CONTROL	HV1	HV2	REC1	REC2
7	21.8	22.4		26.3	
8	15.1	10	16.5	20.3	16.8
9	36.4	22.5	18.5	28.1	24.9
mean	24.4	18.3	17.5	24.9	20.8
SD	10.9	7.2	1.4	4.1	5.7

Measurements are in LDF units. HV1, first four minutes of hyperventilation, HV2, second four minutes of hyperventilation, REC1, first four minutes of recovery, REC2, second four minutes of recovery. Subject 7 undertook hyperventilation for only four minutes due to bronchoconstriction, and the bronchoscope was removed after four minutes recovery period.

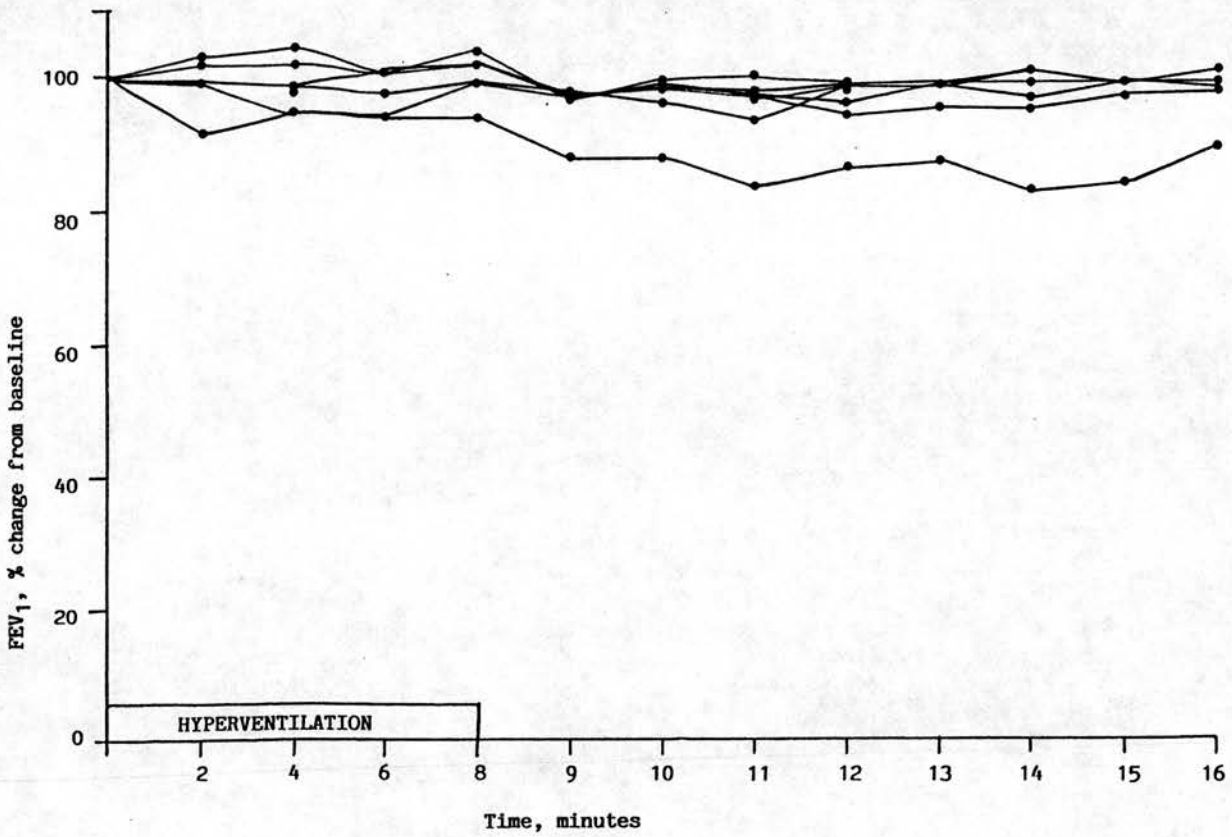


Figure 7.4. Change in airway calibre during and after cold air hyperventilation, expressed as % change from baseline, in non-asthmatic subjects (n = 6).

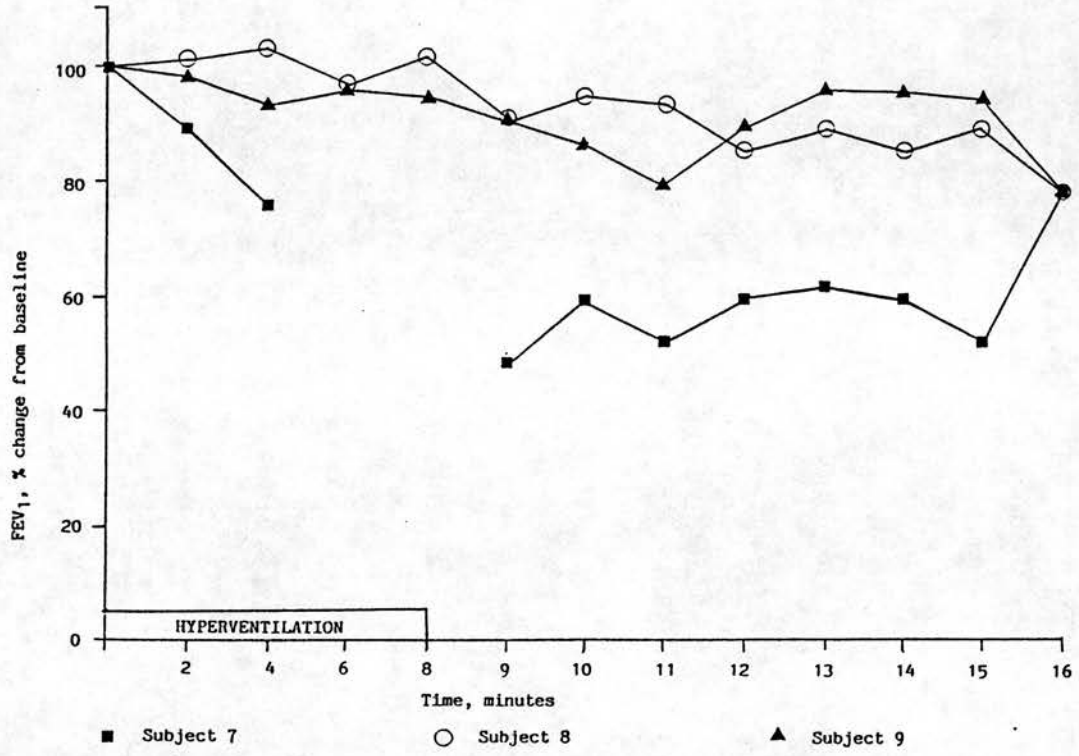


Figure 7.5. Change in airway calibre during and after cold air hyperventilation, expressed as % change from baseline, in asthmatic subjects (n = 3). Subject 7 stopped hyperventilation after 4 minutes due to bronchoconstriction, the fall in FEV1 exceeding 15% form baseline.

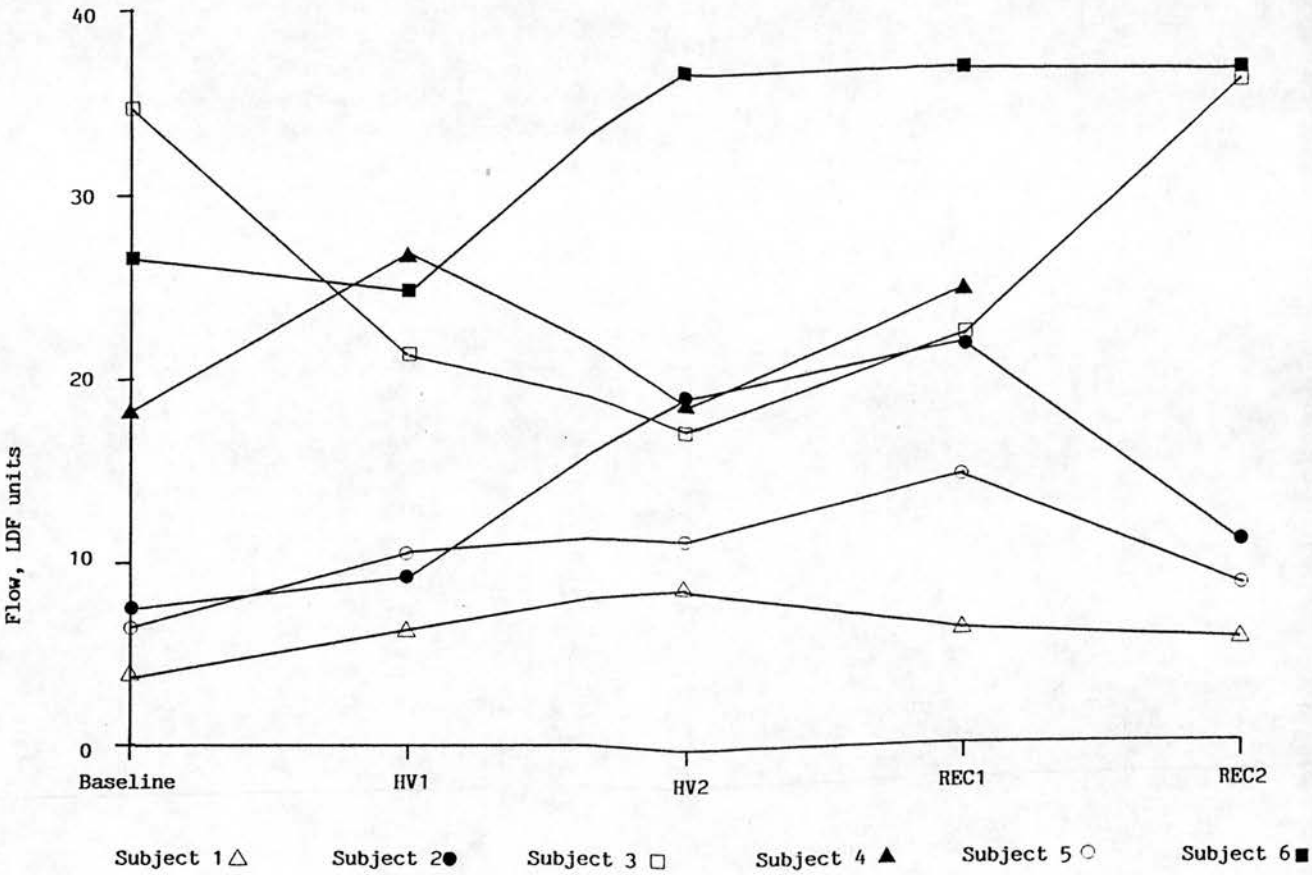


Figure 7.6. LDF measurements during baseline conditions, hyperventilation of humid air, and recovery in non asthmatic subjects. HV1, first four minutes of hyperventilation, HV2, second four minutes of hyperventilation, REC1, first four minutes of recovery, REC2, second four minutes of recovery. In subject 4, the bronchoscope was removed after the first four minutes of recovery due to discomfort.

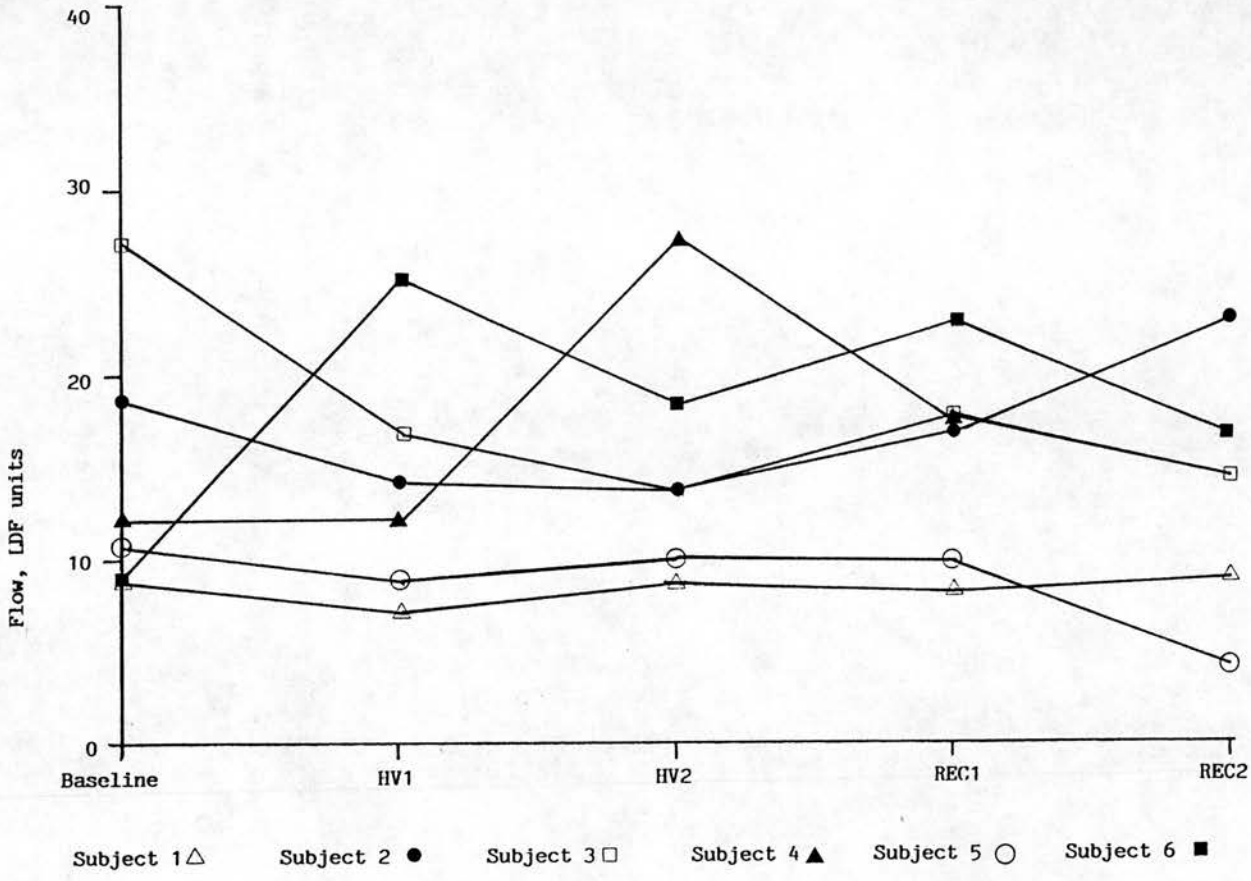


Figure 7.7. LDF measurements during baseline conditions, hyperventilation of cold air, and recovery in non asthmatic subjects. X-range labels as in figure 7.6.

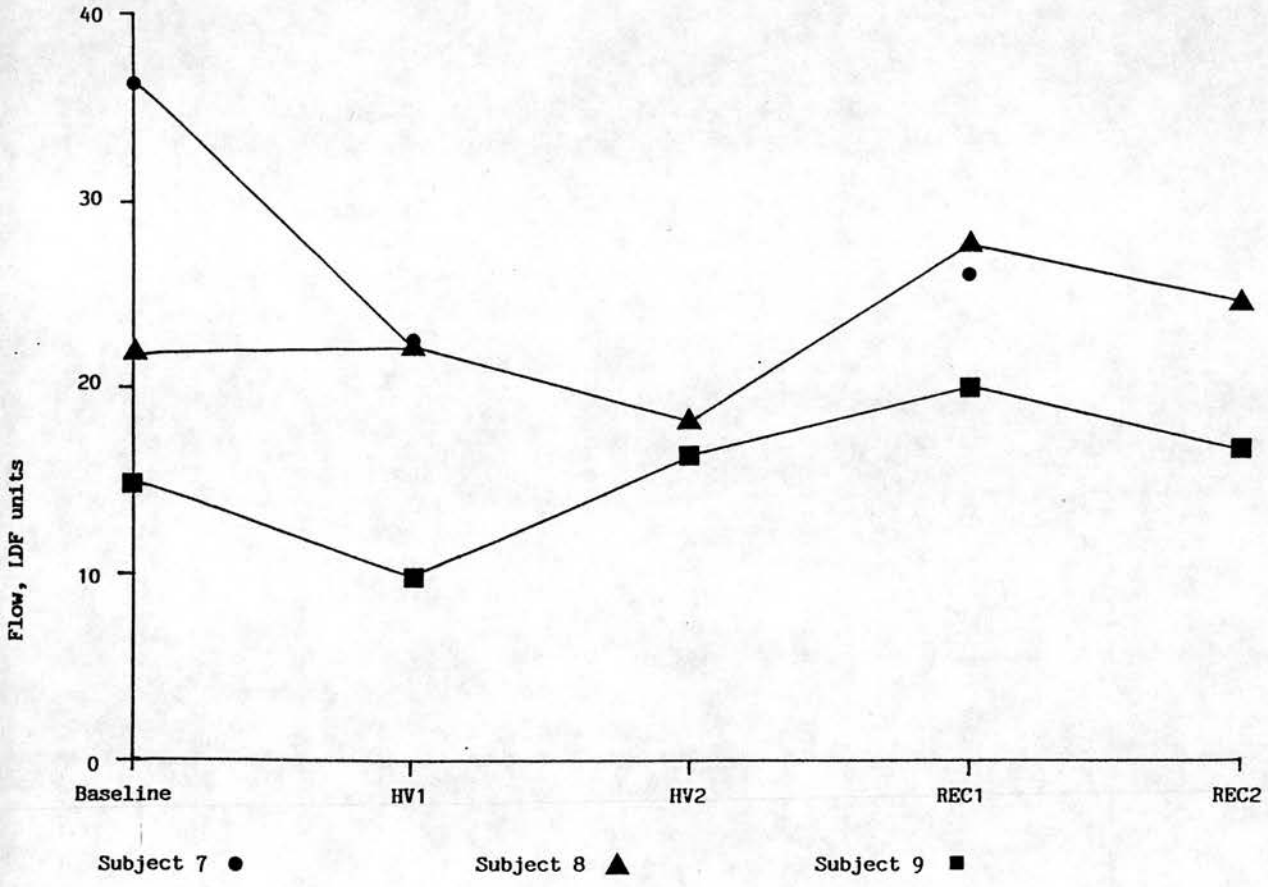


Figure 7.8. LDF measurements during baseline conditions, hyperventilation of cold air, and recovery in asthmatic subjects. X-range labels as in figure 7.6. Subject 7 stopped hyperventilation after 4 minutes, and the bronchoscope was removed after the first four minutes of recovery due ti discomfort.

7.6 DISCUSSION

The results of this study demonstrate that it is feasible to obtain LDF measurements in the trachea of spontaneously breathing subjects, without additional complications or discomfort other than that associated with bronchoscopy. However, the interpretation of the LDF signals is difficult. Many of the characteristics of the measurements and sources of error and artifact were similar to those already observed in the animal studies. Artifacts associated with ventilation and cardiac motion were observed, and it was therefore necessary to make measurements during breatholding and to choose sites where cardiac motion was minimal. It was also necessary to administer atropine to reduce airway secretions, in order to achieve adequate contact of the probe with the mucosa.

The site-to-site variation observed in LDF measurements during baseline conditions in the awake humans was substantial, though less than that seen in sheep and dogs in the earlier studies. Site-to-site variation was of similar degree in subjects undergoing cardiopulmonary bypass, in whom there was no motion artifact. This suggests that variation in LDF signals does reflect true local variation in perfusion rather than motion artifact. The variation between regions observed in the cardiopulmonary bypass patients was similar to that observed in the sheep described in chapter 5. This will be further dicussed in chapter 8.

It has been proposed that the pathogenesis of exercise induced asthma may involve vasoconstriction in the airway wall during the period of cold or dry air hyperventilation, followed by vasodilation during the recovery period (Gilbert et al, 1987). The thickening of the airway wall during the latter period might contribute to airway narrowing. Hyperventilation of cold and dry air has been shown to

increase airway wall blood flow in animals in a number of studies (Baile et al, 1985, 1987 a and b, Parsons et al, 1988). Due to the small numbers of subjects in the present studies and the difficulty in interpreting the LDF signals, no definite conclusions can be drawn regarding the effect of cold and humid air hyperventilation on blood flow in the airway. It is clear that hyperventilation of cold air did induce bronchoconstriction in the asthmatic subjects, and in one of the non-asthmatic subjects, while airway calibre changes in the other subjects were of minor degree only. LDF signals however showed no systematic change during or after hyperventilation, suggesting that either no uniform change in airway wall blood flow occurred, or that, if it did occur, it was not detected by the LDF probe. In the three asthmatic subjects studied, a small increase in LDF signals in the early part of the recovery period was seen in two subjects, and a fall was observed in the other. A much larger group would have to be studied to fully investigate this question, and in view of the potential risks of bronchoscopy and the uncertainties encountered in interpreting the LDF signals, we did not consider it ethical to proceed with this study until further understanding and improvement of LDF technology has been achieved.

In the study described in chapter 6, in which dogs were hyperventilated with warm dry air, an increase in tracheal blood flow was detected by the reference flow radiolabelled microsphere technique, but not by the LDF technique. Thus it is possible that an increase in blood flow did occur in this study and was undetected. Alternatively, the drugs necessary to perform bronchoscopy may have attenuated any rise in blood flow. Administration of atropine is unlikely to have influenced the blood flow response, since, as discussed in chapter 1, cholinergic blockade has little effect on

resting tone in the airway vasculature, and in other studies, cholinergic blockade did not influence the increase in airway blood flow occuring in dogs in response to dry air hyperventilation (Baile et al, 1986). In other studies, however, it has been shown that this increase in blood flow is attenuated by the topical administration of lignocaine (Baile et al, 1988). In the present study, sufficient topical lignocaine was administered to prevent coughing, and this may have prevented an increase in tracheal blood flow.

Thus, although it has been demonstrated that LDF measurements can be made in the trachea of human subjects, and that they share the characteristics of measurements obtained in animals, the difficulties in interpreting the meaning of the signals obtained indicate that further technical developments and animal studies will be necessary before the technique can be meaningfully applied in humans.

CHAPTER 8

GENERAL DISCUSSION

Laser Doppler flowmetry is a technique designed to measure microcirculatory blood flow which should provide real-time measurements obtained in a non-invasive fashion. The technique has been evaluated and used with success in many biological systems, most notably the skin (Holloway, 1983) and gastrointestinal system (Shepherd & Riedel, 1982, Ahn et al, 1986a.b.c). The present studies were designed to examine the use of laser Doppler flowmetry, applied in a manner similar to that used in the gastrointestinal tract (Granger & Kvietys, 1985), to measure blood flow in the central airways. A number of characteristics of LDF measurements have been demonstrated in the airways of dogs, sheep and humans. Some sources of error and artifact have been identified, although several aspects of the signals obtained remain unexplained. The results indicate that acute changes in blood flow may be demonstrated in real time when the probe is held at a single recording site in the airway. The magnitude of change of LDF blood flow signal at a specific site within the airway may be less than the magnitude of the corresponding overall change in airway blood flow. This may be a result of technical factors related to LDF measurement or to autoregulation occurring in the microcirculation, and this requires further investigation. If the LDF probe is moved between sites during an intervention, the response measured by LDF may differ both quantitatively and qualitatively from blood flow measurements obtained by the radiolabelled microsphere reference flow technique. This suggests that laser Doppler flowmetry cannot be used in this fashion to provide an accurate reflection of overall airway wall blood flow. Further modifications of the LDF technique may however improve the accuracy of measurements. In this section, results from other biological systems are reviewed in relation to the present studies, and possible future modifications to

improve accuracy of LDF measurements in the airways are discussed.

Site-to-site variation in laser Doppler flow signals is a characteristic of LDF measurements in all biological systems. This variation has been considered to reflect true differences in blood flow (Johnson et al, 1984), either related to variations in capillary density, such as those occurring in skin (Landis, 1938), or to local physiological variations in flow, as a result of autoregulation or possibly microvascular steal phenomena (Kiel et al, 1987). Table 8.1 summarises the variance of laser Doppler flow signals between sites observed in other species and organs, and, for comparison, shows the variation between sites, between regions, and between individual subjects for all three species in the present studies. Variance between regions is also shown for the reference flow technique. For the airway measurements, the variance between sites is lower in the humans than in the animals. In the humans, the variance between sites is similar in both the anaesthetised subjects during cardiopulmonary bypass, in whom potential artifacts due to repiratory or cardiac motion are absent, and in the awake subjects during breath-holding. The variance between regions and between subjects is substantial in all species, and, in dogs, is similar for both the LDF technique and the reference flow technique.

TABLE 8.1 VARIABILITY IN LASER DOPPLER FLOW MEASUREMENTS IN ALL SPECIES

	CV (sites)	CV (regions)	CV (subjects)	Reference
Sheep	57 (26)	47 (21)	31 (27)	Ch 5
Dogs				
LDF	53 (19)	35 (8)	54 (14)	Ch 6
Reference flow	NA	39 (17)	52 (10)	Ch 6
Humans				
Non-Asthmatic	23 (14)	NA	71	Ch 7
Asthmatic	31 (18)	NA	45	Ch 7
Cardiac Bypass	18 (11)	51 (16)	54	Ch 7
Skin	25		Swanson Beck	1986
Small Intestine	15		Ahn	1986a
Stomach	33-39		Kvernebo	1986
Rats				
Renal Cortex	9 (1)			
Gracilis muscle	34 (4)		Smits	1986
Cremaster	47 (5)			

Values shown are coefficient of variation (CV), displayed as mean (SD).

These observations support the idea that the variation in LDF signals does reflect true local variations in blood flow in the airway. Observations from other studies indicate that site-to-site variation in LDF values is least in renal cortex (Smits et al, 1986) and greatest in skin (Johnson et al, 1984) (Table 8.1).

This local variation in flow, combined with the high spatial resolution of the laser Doppler technique, provides one of the major problems in attempting to apply laser Doppler flowmetry to reflect blood flow to a whole organ or part of an organ. In the present studies, several sites within a region of the airway were sampled sequentially, and the measurements were averaged in an attempt to provide a value representative of that region. However, the data shown in chapter 6 indicate that, even when an average measurement was derived from twelve sites in each region, the resulting value did not accurately reflect regional blood flow when compared to radiolabelled microsphere reference flow measurements. Furthermore, as is demonstrated in the study in sheep (Chapter 5), even when the probe is held at a single site during a series of interventions to alter blood flow, the behaviour of the microvasculature at that site may differ profoundly from that at other sites within the same organ, or from the organ as a whole. In an attempt to deal with the spatial variation in flow signals in skin, Salerud and Nilsson (1986) developed a probe which sampled from seven sites simultaneously and provided an integrated flow signal. Theory would suggest that the spatial variation should be reduced by $\sqrt{7}$.
and this indeed was the case. Development of an integrating probe sampling multiple sites for use in the airway would therefore be a logical step in the future application of this technique. This would also reduce the sampling time necessary to obtain representative flow

signals from an airway region.

In addition to spatial variation in LDF signals, several authors have observed temporal variations in signals. Slow, rhythmic oscillations in LDF signals, occurring at 4 - 7 cycles/minute were observed in rat gastric mucosa by Holm-Rutili et al (1986). They confirmed that these signals were related to cyclical changes in blood velocity by the simultaneous use of intravital microscopy to directly observe the microcirculation. Kiel et al (1985) observed oscillations of similar frequency in LDF signals measured in canine gastric mucosa. They initially thought that these were artifactual due to contraction of the muscularis, but in a subsequent study (1987) they found that the oscillations were abolished when the microcirculation was perfused with a cell free solution, and thus concluded that the oscillations did reflect movement of red cells. Damber et al (1986), studying rat testis, also observed cyclical changes in LDF signals, this time at the slightly higher frequency of 6 - 10 cycles/minute. They again demonstrated, by the use of intravital microscopy, that these signals were due to cyclical variation in microvascular haematocrit and red cell velocity. Kvernebo et al (1986) observed similar fluctuations in LDF signal in human gastric mucosa, as did Olsson and Bende (1986) in human nasal mucosa. The latter authors found that this "vasomotion" could be abolished by stellate ganglion blockade, indicating that it is under sympathetic neurogenic control. It has been suggested that this vasomotion, or cyclical flow in the microcirculation, facilitates fluid exchange between the microvasculature and the interstitium by altering the balance between hydrostatic pressure in the vessels and tissue pressure in the interstitium (Intaglietta, 1981). A criticism of the design of the present studies would be that, if such vasomotion occurred in the microvasculature of the airway, and at a similar

frequency, then our sampling time of about five seconds would be inadequate to detect this, and a significant proportion of the variance between measurements might be attributable to vasomotion. We used a relatively short sampling time as ventilation had to be temporarily stopped to avoid respiratory movement artifact, and we wished to keep the period of cessation of ventilation to a minimum to avoid the development of hypoxia or hypercapnia which would alter airway blood flow, as discussed in chapter 1. This problem will therefore require investigation with a different protocol, particularly since it may be even more important if an integrating probe sampling multiple sites is used. Use of such a probe reduces spatial variation, but increases temporal variation in signals (Salerud & Nilsson, 1986).

The next area of uncertainty in the use of LDF relates to the depth of measurement within the tissue of interest. The theory of laser Doppler flowmetry devised by Bonner and Nossal(1981) suggests that measurements should be derived from a tissue volume of $1mm^3$. This however has proved difficult to test in biological systems, and conflicting results have been derived by different workers. Studies in isolated loops of bowel have provided a useful model to examine this problem, since LDF probes can be applied to either side of the bowel wall, and the effects of interventions studied. Using this type of preparation, Shepherd and Riedel (1982) found that reactive hyperaemia occurring in the mucosa of canine small bowel was detected from the mucosal, but not the serosal side. Furthermore, using selective vasodilators of the mucosa and the muscularis, and measuring total blood flow into the segment using an electromagnetic flow probe, they were able to demonstrate differential responses in blood flow to the mucosa or muscularis externa measured by LDF compared to total

blood flow. They concluded that the LDF probe derived its signal from only a superficial portion of the bowel wall. In a series of further studies, they demonstrated differential responses in mucosal versus muscularis flow in response to instillation of glucose and bile into canine ileal loops (1985), and different degrees of autoregulatory escape during sympathetic stimulation in mucosa compared to muscularis (1988). In all of these studies, they showed that LDF measurements made from the mucosal side differed from those made from the serosal side, although the precise depth of penetration could not be determined. Representative measurements of bowel wall thickness in one of their studies (1984) indicated that the mucosa was 1.47mm thick, the muscularis mucosa, 0.27mm, the submucosa, 0.44mm, the muscularis externa, 1.44mm, and the serosa, 0.14mm.

In a similar series of experiments from another group, however, differing conclusions were obtained. Ahn et al (1985) initially studied isolated loops of feline small intestine, obtaining LDF readings from the mucosal and serosal aspects and measuring total blood flow by an optical drop-recorder method. They found that high correlations existed between total blood flow and LDF signals derived from either side of the bowel wall. In a similar study in humans (1986a), they again demonstrated LDF signals of equal magnitude when measurements were obtained from either side of the bowel wall. When the measurement was derived from the serosal surface, and a mirror was placed in the lumen opposite the probe, the LDF signal doubled, on average. This was considered as evidence that the laser light was traversing the entire thickness of the wall, although this observation alone cannot be considered as evidence that the blood flow measurement under normal circumstances is necessarily derived from the full thickness of the bowel wall. Similar observations were again made by

the same authors in segments of human colon studied intraoperatively (1986b). In these studies, the thickness of the bowel wall was found to vary from 4.3mm to 5.9mm, and the authors therefore concluded that the depth of measurement could be up to 5.9mm.

Another approach to this problem has been to interpose layers of unperfused tissue between the probe tip and the tissue of interest and to examine attenuation of the LDF signal. Results again differ between studies. Holm-Rutili et al (1986), studying rat gastric mucosa, found that by interposing a 1mm layer of unperfused tissue, the LDF signal could be reduced by 90 - 95%. Kiel et al (1985), found that interposing a layer of unperfused tissue of unspecified thickness reduced LDF signals in their canine stomach model by 85%. In contrast, Kvietys et al (1985), studying feline jejunum, found that although interposition of a 3mm layer of unperfused tissue between the 1DF probe and the mucosa under study reduced absolute LDF values by about 80%, changes in blood flow in the underlying tissue could still be reliably detected. They concluded therefore that their LDF probe measured to a depth of at least 3mm. Johansson et al (1987), studying feline small intestine, interposed layers of unperfused tissue up to 6 mm in thickness, and found the the LDF signal decayed in an exponential manner, with 23% of the signal persisting when the 6mm layer was interposed. From mirror experiments similar to those described above, they estimated that the largest measuring depth of their instrument was 6mm.

The reasons for these differing findings are not entirely clear. It has been suggested that instrumental factors such as the power output and optical fibre diameter within the probe might explain the different tissue penetration (Granger and Kvietys, 1985). The instrument used by Shepherd and colleagues, for example, has a greater

power output, but a smaller fibre diameter than than that used by Ahn and colleagues. The instrument used in the present studies has a power output of 1.6mW, and its depth of measurement within the airway wall cannot be determined from the present studies. If the measurement were derived only from the airway mucosa, then it would be expected that the correlation between the LDF measurements and the mucosal blood flow values determined by the reference flow technique would be greater than that between the LDF values and the total wall blood flow value. However, the r^2 values for each of these regressions under each set of conditions are not significantly different as shown in table 8.1. This might suggest that the LDF measurement is not derived purely from the airway mucosa, but, since the correlations are all relatively poor, a definitve statement cannot be made.

TABLE 8.2 r^2 VALUES - LDF vs REFERENCE FLOW MEASUREMENTS

	MUCOSA	TOTAL WALL
BASELINE	0.18	0.19
HYPERVENTILATION	0.16	0.19
PEEP	0.5	0.54

In addition to uncertainty about the depth of measurement of laser Doppler flowmetry in the airway wall, there is a further problem in that the thickness of the mucosal and submucosal components of the airway wall may vary considerably from site to site (Laitinen et al. 1986b), and the LDF measurements are tissue volume dependent. This problem has been addressed in studies of intestinal blood flow in which the thickness of the intestinal wall at each site of measurement was determined and the LDF signals were corrected for wall thickness (Ahn et al, 1986b). However, attempts to determine wall thickness in the airway negate the non-invasive advantage of laser Doppler flow

measurements. In addition, it is known that mucosal thickness varies not only from site to site, but may also vary over time at individual sites as vascular resistance changes (Laitinen et al. 1986b). In general, vascular resistance and thickness of the mucosal/submucosal component of the airway wall vary inversely. Thus any form of correction of the laser Doppler flow signals for airway wall thickness would require a dynamic method of measuring mucosal/submucosal thickness which provided measurements in real time. Such a development is currently impractical.

The next problem in applying LDF for airway blood flow measurement is to identify and isolate sources of signal other than that related to blood flow, and it is in this area that the most important difficulties arise. Since the LDF probe is basically a sensitive movement detector, then any source of movement within its sampling volume will generate a signal. Whether the movement is classed as "signal" or "artifact" depends on the parameter which the investigator wishes to study. Use of laser Doppler flowmeters in all biological systems has been associated with the detection of "flow" signals unrelated to microvascular blood flow (Stern et al. 1977, Ahn et al,1987).

Several potential sources of signal unrelated to blood flow have been identified in the airways in the present studies. Movement of the airway wall associated with ventilation caused major artifacts which were easily eliminated. Movement of the airway wall due to transmitted pulsation from the heart and great vessels caused major artifacts in some regions of the airway, and was probably an important contributor to the "noise" observed in the sheep study (Chapter 5). Perfusion of the bronchial artery with a cell-free solution in that study suggested that "noise" accounted, on average, for 20% of the

total LDF signal under baseline flow conditions. In a recent study examining the use of laser Doppler flowmetry to study myocardial perfusion in the empty beating porcine heart, Ahn and colleagues (1988) have encountered similar problems. In their preparation, they measured myocardial perfusion using a laser Doppler flow probe on the epicardium and another LDF needle probe inserted into the myocardium. The animals were maintained on cardiopulmonary bypass during the procedure, but the heart was allowed to beat. LDF signals during stepwise increases of the extracorporeal blood flow rate, which would alter myocardial perfuson via coronary collateral vessels, were compared with coronary sinus flow rates measured directly. They found significant linear correlations between LDF signals and coronary sinus flow rate. However, by plotting LDF versus coronary sinus flow, and extrapolating to zero values for coronary sinus flow, they found that the residual LDF signal at zero flow varied from 2.24% to 60.9% of the highest flow observed in each experiment. Since there were no sources of collateral blood supply to account for this signal, they concluded that this signal represented the contribution of "noise" due to movement of the preparation. The level of "noise" varied, not only between animals, but between occasions within the same animal, leading to difficulties in interpreting the significance of changes in laser Doppler signal. In the present studies in humans (chapter 7), the observation that the actual LDF signal was similar in subjects in whom the heart was beating, and in those in whom it was arrested, i.e. those on cardiopulmonary bypass, suggests that the contribution of transmitted cardiac motion to LDF signals measured in the airways of humans may be relatively small, but it must remain a significant source of error in the measurements.

In situations in which major movements of the tissue under study

occur, there is also potential for movements of the probe itself giving artifactual signals due to movements of the optical fibres. This problem was encountered by Newsom et al (1987) who were attempting to monitor foetal scalp blood flow during labour. They considered several methods of reducing the problem, including the use of laser diodes, mounted at the "probe" tip, and therefore avoiding the need for optical fibres, the use of monomodal fibres, which reduce the number of possible modes of propagation, the use of a modified spectral analysis, and finally the use of a reference fibre which derives a signal from a target within the transducer, and therefore defines the signal due to movement, allowing this to be subtracted from the flow signal. This latter technique provided potentially the most effective solution, and a similar technique was employed successfully by Ahn et al (1988) in their studies of myocardial perfusion in the beating heart. It may be appropriate to include such a fibre in the future design of a probe for use in the airway.

Apart from these gross movements, a number of other sources of movement occur in the airway which may give rise to LDF "flow" signals. Ciliary motion is detected by the LDF probe, but, as described in chapter 3, probably contributes only a small amount (about 2 %) to the LDF signal at physiological rates of flow. Motion of the vascular walls and the surrounding tissue as a result of blood flow through the microvascular bed may also contribute to the flow signal. In a study in cats, Martling et al (1987) found that it was necessary to pretreat the animals with atropine to abolish artifacts related to smooth muscle contraction. In all of the present studies, pretreatment with atropine was used, although this was done principally to reduce airway secretions which we found to be an important cause of inadequate contact betwwen the LDF probe and the

mucosa.

Each of these sources of "noise" will become quantitatively more important during conditions of low blood flow, and the tendency for laser Doppler flowmetry to overestimate tissue perfusion at low levels of perfusion, and to detect a signal even at zero blood flow, is well recognised in all biological systems (Stern et al, 1977, Ahn et al, 1987). Any or all of the above factors might be important in the genesis of this signal. The practical implication of this finding, also demonstrated in the present studies (Chapter 5), is that, even with further modifications, use of LDF may be limited to qualitative, rather than quantitative measurement of blood flow. If quantitative measurements are to be obtained, an independent calibration within each species, and even within each individual subject and organ will be necessary.

Another particular problem in interpretation of the laser Doppler signals in the airways relates to the complex nature of the airway microvascular bed, and, specifically, to the multiple sources of inflow to, and outflow from, the microvascular bed, and the lack of an appropriate "gold standard" for measuring microvascular flow in the airway wall. As discussed in chapter 1, inflow to the airway vascular bed may be from a variety of systemic vessels. The extent of the collateral blood supply to the trachea is evidenced by the observation that extensive surgical devascularisation of the trachea is necessary before tracheal tissue necrosis occurs (Fujita et al, 1988). In addition, a proportion of airway blood flow may be derived from the pulmonary vasculature (Barman et al, 1988). It has been suggested that in dogs, the latter component may provide as much as 50% of the bronchial wall blood flow, though only about 3% of the tracheal blood flow. In guinea pig lungs, substantial perfusion of the trachea via

the pulmonary artery has been demonstrated in an isolated whole lung preparation using Evans blue dye and fluorescein isothiocyanate dextran (FITC-D) (Kroll et al, 1987). It is not known whether the pulmonary circulation provides a contibution to airway blood flow in sheep or humans. The physiological importance of the collateral sources of blood supply to the trachea is also evidenced in the studies of Laitinen et al (1987b). In their preparation in dogs, one branch of the superior thyroid artery was isolated and perfused, and pressure in this vessel was monitored. When perfusion in the vessel was set to zero, a significant pressure, up to 80 mmHg, persisted. This "stop-flow" pressure is derived from the collateral vessels.

The situation is further complicated by the fact that flow through anastomotic or collateral vessels is pressure-dependent. The present studies in sheep (Chapter 5), for example, suggest that potential anastomoses between tracheal and bronchial microcirculations exist, but are only perfused when an imbalance in perfusion pressure between the tracheal and bronchial systems develops. Similar considerations will apply to anastomoses between the systemic and pulmonary systems. The relative contributions of the various anastomotic or collateral vessels will therefore vary as the balance of pressures in the different systems varies. The existence of such anastomoses raises difficulties in comparing the laser Doppler method with other methods of measuring blood flow. In the studies in sheep (chapter 5), anastomotic flow may account for the observation that changes in airway blood flow detected by the LDF system do not scale with changes in blood flow through the bronchial artery. In the studies in dogs (chapter 6), the radiolabelled microsphere measurements should reflect all systemic blood flow to the airway, irrespective of the source of inflow, but would not reflect pulmonary

blood flow to the airway. Thus both of the standards with which LDF is compared in the present studies have limitations dictated by the complexity of the vasculature.

Even if a standard technique could be designed which measured all sources of inflow to, and outflow from, the airway wall vessels, it is still possible that redistributon of blood may occur within the airway wall without net flow occurring. Since LDF measures red cell flux rather than flow, this type of movement would result in generation of an LDF signal.

Finally, the differential responses of the circulation in the airway mucosa/submucosa and in the remainder of the airway wall to various stimuli pose another difficulty. In the present studies, hyperventilation of dry air induced an increase in airway mucosal blood flow as measured by the reference flow technique in dogs, but no consistent change in blood flow to the airway cartilage or adventitia was observed. This differential response to cold or dry air hyperventilation has been previously noted by Baile et al (1987a), and a similar response to histamine infusion in sheep has been reported by Kramer et al (1988). In pigs, infusion of neuropeptides may produce decreases in vascular resistance in the superior laryngeal artery or the bronchial artery, without producing changes in laser Doppler flow signals in the mucosa in the distribution of these vessels (Matran, 1989b), again suggesting differential responses.

A further confounding variable may be the occurrence of autoregulation. To date, there is no definite evidence that the airway vasculature is capable of autoregulation. However, data from the present study in sheep (Chapter 5), suggests that autoregulation might occur. In dogs, using a chambered stomach flap preparation, Kiel et al (1987), have demonstrated that autoregulation of gastric

mucosal blood flow does occur over the range of systemic pressure from 90-150 mmHg. It has been suggested that this might be important for fluid tissue balance. If maintenance of airway fluid balance is an important function of the airway circulation, then it might be expected that autoregulation would occur. This question requires further investigation, and may have an important bearing on studies comparing LDF measurements with measurements of total flow to any organ.

It was hoped at the outset of the present studies that LDF might prove a useful method of measuring airway wall blood flow in humans. The results of the animal studies to date however indicate that interpretation of results obtained using LDF in human studies is still open to question. In addition to the problems identified in the animal studies, use of the technique in awake human subjects is associated with further practical problems. Although the laser Doppler flowmeter provides a real time signal which is derived non-invasively, the introduction of the probe via the bronchoscope necessitates administration of topical anaesthesia, which may itself alter vascular tone and responses of the vasculature to stimuli thought to be mediated via sensory peptidergic nerves. It has been shown for example that, in dogs, the vasodilation of airway vasculature in response to hyperventilation of cold air is attenuated by topical administration of lignocaine (Baile et al, 1988). In the present studies, it was also clear that the presence of secretions in the airway lumen interfered significantly with the LDF signals, and administration of the anticholinergic agent, atropine, was therefore necessary to prevent accumulation of secretions. As discussed in chapter 1, the cholinergic system, although it has major effects on tracheal and bronchial smooth muscle tone, has been found in previous

studies to have little influence on resting vascular tone in the trachea (Laitinen et al, 1987c, Sahin et al. 1987.). Furthermore, cholinergic blockade does not alter the airway vascular response to hyperventilation of cold air in dogs (Baile et al, 1986). However, the role of the cholinergic system in other reflex responses of the airway vasculature is unknown, and the need to administer atropine therefore further complicates the interpretation of blood flow data obtained during interventional studies.

In summary, the present studies indicate that while the laser Doppler flowmeter introduced via a bronchoscope can detect acute changes in blood flow within small, incompletely defined, areas of the airway wall, it can provide only qualitative measurements, and it cannot be used to assess regional changes in blood flow. If the technique is to be further developed for use in the airway, a number of modifications of the LDF instrument will be necessary. These will include development of a probe capable of sampling multiple sites simultaneously, possibly incorporating a reference fibre to correct for fibre movement artifact. One attractive proposition is to develop a hybrid probe, such as that designed by Diresta et al (1987). They incorporated, within a single probe, optical fibres permitting laser Doppler flow measurements and platinum electrodes allowing local generation of hydrogen ions in the tissue and measurement of blood flow by the hydrogen clearance method. This system therefore combines the advantages of the LDF system, providing continuous measurements of tissue perfusion, with those of the hydrogen clearance method which derives a quantitative blood flow signal expressed per unit mass of tissue. The hydrogen clearance measurements are discontinuous, but can be made intermittently and used to calibrate the LDF system. Using such a system in an isolated canine gastric preparation, the

authors were able to demonstrate that the relationship between measurements made by the two methods was linear, and highly significant ($r = 0.94$). The major problem in constructing such a probe for use in the airway would be fragility of the platinum electrodes.

Laser Doppler flowmetry undoubtedly has potential application for the measurement of blood flow within the airways. However, many practical problems remain to be overcome, the most important being movement artifact. Even when these problems have been solved, it is likely that quantitative measurement will continue to require in vivo calibration with another method of measuring microvascular blood flow. The present studies indicate that the technique, as used here, cannot yet be meaningfully used to measure airway blood flow. Nevertheless, the potential ability of the technique to provide continuous, non-invasive measurements of blood flow in humans suggest that further development is indicated.

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APPENDIX I

SPECIMEN CALCULATION OF NUMBER OF MICROSPHERES PER TISSUE SAMPLE

e.g. ^{113}Sn

Specific Activity 358.9 MBq/g 9.70mCi/g

Volume 10mls

Microspheres/mg 3.04×10^5

Milligrams per vial 103.1 mg

If 1 ml is injected, this is equivalent to:

10.31 (mg/ml) $\times$ 3.04×10^5 (spheres/mg)

$= 3.13 \times 10^6$ spheres/ml

If the cardiac output is 2500 ml/min, then the required flow (X) indicating 400 microspheres in the sample is:

$$X = \frac{2500 \text{ (ml/min)} \times 400}{3.13 \times 10^6}$$

$= 0.32$ ml/min

By rearranging the equation, the number of spheres in any tissue sample can be determined, and the stochastic (Relative) error can be calculated from the formula:

$$\text{Relative Error} = \frac{1}{\sqrt{n}}$$

Numbers of spheres and relative errors for all tissue samples are shown in Appendix II.

APPENDIX II

MICROSPHERE MEASUREMENTS: PERCENTAGE ERRORS PER TISSUE SLICE

	CONTROL	HV	PEEP
DOG 1			
	8.6	4.8	4.8
	9.9	8.4	6.5
	12.6	7.8	6.6
	11.0	10.6	5.0
	13.1	13.8	6.3
	4.9	2.8	4.7
	5.1	2.7	3.3
	7.0	8.5	5.4
	12.3	8.8	7.1
	20.4	23.0	12.9
	20.6	15.6	9.3
	4.7	3.3	4.2
	3.8	3.2	4.4
	16.1	16.0	9.1
	16.6	13.1	9.4
	24.9	24.1	11.5
	14.9	15.8	10.1
	5.7	4.6	2.9
	4.7	3.7	3.6
	14.9	13.5	9.9
	15.4	16.2	12.7
DOG 2			
	9.5	3.9	7.9
	11.6	6.2	13.2
	17.8	7.8	14.3
	19.6	11.6	22.7
	15.9	13.8	22.4
	5.2	3.1	5.1
	5.4	3.0	7.3
	8.5	8.0	13.8
	17.8	15.2	21.2
	18.5	19.7	39.4
	29.3	31.1	25.4
	5.6	3.0	5.2
	10.6	3.9	6.3
	21.5	7.8	13.5
	24.8	13.0	18.9
	61.2	19.0	38.6
	40.7	11.7	28.1
	6.0	3.6	7.8
	8.8	4.0	6.8
	12.4	6.4	12.7
	17.6	16.0	12.3

HV, hyperventilation.

	CONTROL	HV	PEEP
DOG 3			
	6.1	3.4	6.6
	9.7	4.8	7.9
	14.4	7.4	12.5
	10.5	11.2	11.4
	10.8	23.3	18.5
	6.8	2.8	5.7
	9.5	3.4	11.5
	7.1	5.9	7.7
	9.4	9.5	13.2
	14.8	21.3	15.2
	16.8	32.4	27.2
	4.8	3.1	6.0
	6.8	3.6	7.4
	11.3	9.2	12.5
	13.5	13.5	16.9
	11.6	13.5	14.3
	11.4	14.0	22.6
	4.8	3.4	5.9
	2.8	3.0	8.9
	9.6	8.6	10.9
	7.7	9.3	14.4
DOG 4			
	5.5	2.3	5.3
	7.9	3.4	5.7
	9.0	5.6	12.2
	8.4	6.1	8.1
	7.9	5.9	7.5
	4.5	2.4	6.9
	4.5	2.3	5.5
	8.8	8.5	11.7
	7.0	7.0	10.2
	14.7	16.6	16.1
	11.8	9.7	6.6
	4.6	2.1	5.1
	3.8	2.1	4.5
	14.0	10.6	17.7
	7.9	4.9	9.4
	17.0	12.8	14.9
	11.5	7.8	8.5
	3.4	2.5	3.9
	3.1	2.2	4.7
	11.7	9.7	8.6
	10.0	7.1	11.4

HV, hyperventilation.

DOG 5	CONTROL	HV	PEEP
	13.0	4.3	8.8
	17.8	6.4	27.1
	26.4	9.3	13.8
	10.7	7.8	7.5
	5.8	5.8	8.3
	9.9	3.3	7.5
	8.3	2.5	7.0
	7.3	14.1	12.4
	5.7	6.0	5.9
	35.2	13.4	9.7
	16.4	8.2	6.5
	9.4	3.5	9.9
	6.1	2.8	6.2
	13.9	9.6	10.2
	9.1	7.3	8.5
	20.3	8.1	18.2
	50.7	21.5	22.2
	8.1	3.7	8.4
	7.4	3.6	9.4
	15.5	13.0	11.7
	10.4	8.2	9.2

HV, hyperventilation.

APPENDIX III

SPECIMEN PRINTOUT FROM SCINTILLATION COUNTER

DATE	TIME	OWNER			BACKGROUND INITIAL	74.7	NO OF SAMPLES 37
JUN5/89	4.25	LD DOG2			FINAL	72.1	
ISOTOPE		TIN 113		RUTHENIUM 103		GADOLINIUM 103	
HALF LIFE (HRS)		2760		936		5808	

SAMPLE NO.	ELPSD.	TIME TEST	ESTIM. COUNTS	ADJSTD. CPM	ERR %	ESTIM. COUNTS	ADJSTD. CPM	ERR %	ESTIM. COUNTS	ADJSTD. CPM	ERR %
1	0.156	125.5	56406	27004	0.3	1397	668	28.4	784	375	32.9
2	0.195	125.4	37220	17803	0.3	686	328	33.11	411	197	35.9
3	0.233	125.4	44792	21424	0.2	712	340	36.6	464	222	36.5
4	0.272	125.6	40109	19160	0.2	481	230	45.7	227	108	63
5	0.31	125.7	-207	-99	48.9	40263	19219	0.6	718	341	21.7
6	0.348	126	-228	-109	44.1	40314	19202	0.6	596	284	25.9
7	0.387	126.2	-276	-131	36.2	39490	18786	0.6	694	330	22
8	0.426	126.1	-298	-142	37.11	41165	19591	0.6	757	360	22.3
9	0.464	125.9	141	67	>100	2323	1108	27.2	88876	42351	0.5
10	0.502	126	86	41	>100	1777	846	35.9	95692	45552	0.4
11	0.541	126.2	230	157	>100	2038	959	38.7	118359	56267	0.4
12	0.58	126	124	59	>100	1615	769	41.4	92521	44537	0.5
13	0.518	125.2	12	6	31.5	45	22	19.7	43	21	13.6
14	0.656	125.5	6	3	64	44	21	20.2	54	26	10.6
15	0.695	125.4	5	2	75.4	33	16	27.3	57	27	10.3
16	0.733	125.7	9	4	39.7	44	21	19.6	45	21	12.5
17	0.772	125.3	4060	1939	1.2	10712	5117	1	4934	2356	1.5
18	0.81	125.7	2711	1298	0.9	4212	2018	1.4	1758	846	2.1
19	0.848	125.6	1160	554	2	2691	1285	2	1507	719	2.4
20	0.887	125.9	959	458	1.7	1213	580	3.2	596	285	4.3
21	0.964	126.2	1458	693	1	645	407	4.2	613	295	3.7
22	1.002	126	13454	6400	0.7	17441	9301	1.3	11812	5619	1.3
23	1.041	125.3	12867	6129	0.7	18516	8824	1.1	5735	2731	2.3
24	1.079	125.8	5098	2432	0.6	2541	1215	2.9	1621	373	3.3
25	1.118	125.8	1160	553	1.3	701	385	4.9	587	328	3.3
26	1.156	125.9	1082	516	1.3	420	200	8.1	198	95	11.1
27	1.194	125.4	429	205	2.2	167	80	13	480	229	2
28	1.233	125.7	11768	5618	0.8	17903	8552	1.2	11592	5533	1.2
29	1.271	125.9	3289	1568	1.7	16562	5039	1.2	7706	3673	1.1
30	1.31	125.6	795	380	2.9	2850	1267	2	1704	314	2
31	1.348	125.4	596	285	2.5	953	456	2.7	860	411	2.6
32	1.387	125.3	98	47	3.2	448	215	4.2	207	99	5.9
33	1.425	125.5	222	106	6.3	1192	570	2.8	391	187	5.3
34	1.468	125.7	10714	4857	0.7	12993	5920	1.3	5047	2409	2.1
35	1.502	126	4805	2289	1.2	10318	4919	1.3	5751	3218	1.8
36	1.54	125.9	2404	1146	1.2	4014	1916	1.7	1913	912	2.3
37	1.579	125.7	1190	569	1.5	534	203	5.9	2044	975	1.4